EFFECT OF LITTER SIZE, DIETARY PROTEIN CONTENT, EWE GENOTYPE AND SEASON ON MILK PRODUCTION AND ASSOCIATED ENDOCRINE AND BLOOD METABOLITE STATUS OF EWES.

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I hereby declare that I have composed this thesis myself, and, except where otherwise stated, the work contained herein is my own.

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ABSTRACT

In a series of experiments, ewe milk production and associated plasma hormone and blood metabolite status were investigated.

Separate comparisons were made between ewes suckling either single (S ewes) or twin (T ewes) lambs, ewes lambing in either January or April, ewes fed either 150 g (low) or 210 g (high) of crude protein/kg DM in the diet and between ewes of the East Friesland (EF) and Scottish Blackface (SBF) genotypes.

In all comparisons milk yields, live weight, body condition score changes, fat, protein and ash contents and energy values of milk were determined weekly. Blood samples were collected on one day each week, at 20 minute intervals for 2 hours, prior to feeding. Samples were pooled within each week and each animal. Plasma glucose, non-esterified fatty acids (NEFA), 3-hydroxybutyrate (3-OHB), urea, albumin, protein, insulin, growth hormone (GH), cortisol, prolactin, triiodothyronine (T3) and thyroxine (T4) concentrations were determined. During weeks 2, 4 and 10 (and 14; genotype comparison only) of lactation blood samples were collection at 20 minute intervals for 8 hours and individually assayed for plasma insulin, GH, cortisol and prolactin concentrations.

Ewes rearing twin lambs had higher milk yields than those rearing single lambs. This was associated with higher NEFA, 3-OHB, GH and cortisol concentrations and lower insulin concentrations in the plasma of twin-rearing ewes. There were no consistent differences in prolactin or thyroid hormone concentrations of the plasma of single and twin rearing ewes. The decline in milk production with advancing lactation was associated with an increase in the post prandial insulin:GH and insulin:cortisol ratios and T4 levels and a decrease in prolactin levels. Feeding was followed by significantly higher insulin levels and slightly higher GH levels.

Ewes fed the high protein diet had higher milk yields and milk protein contents compared with ewes fed the low protein diet. This, however, was not apparently associated with a higher degree of adipose tissue mobilisation. There were no significant differences between the two protein treatments in any of the plasma hormones measured. Change in milk production with stage of lactation was associated with an increase in the insulin:GH and insulin:cortisol ratios and in circulating T4 levels, and a decrease in plasma prolactin levels. During early lactation, feeding was followed by a rise in insulin and GH levels but during late lactation only insulin levels increased.

In contrast to previous observations there was no difference in milk yield or pattern of milk production between the ewes of the EF and SBF genotypes. SBF ewes produced milk of higher fat content compared with EF ewes. All ewes gained similar amounts of live weight throughout lactation. However, plasma albumin and protein levels were lower in EF compared with SBF ewes; plasma insulin, cortisol, prolactin and T3 levels were consistently lower in EF than in SBF ewes. The decline in milk production in late lactation was associated with an increase in the insulin:GH ratio and in T4 levels while prolactin levels decreased. Feeding was followed by increased insulin levels and cortisol levels (EF ewes only) and lower GH levels (during late lactation).

The role of these hormones in the control of milk production is discussed and in particular the hormonal inter-relationships in relation to level and pattern of milk production and associated nutrient status throughout lactation.

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CHAPTER 1

INTRODUCTION

Maternal milk production is the only source of nutrients for the newborn lamb and is the major determinant of lamb growth rate during its early life. Factors which limit milk production in ewes have been investigated; previous work at H.F.R.O. has characterised the effects of differences in lamb suckling intensity, ewe nutrition and ewe and lamb genotype on the level and pattern of milk production (Peart, 1982), and these factors have been used to successfully manipulate milk yield (Doney and Munro, 1962; Peart, 1970a; Peart, Doney and Smith, 1979; Doney, Peart and Smith, 1981). In general manipulation of milk yield using such factors is only effective during early lactation with very little improvement being achieved following peak production (Peart, 1970b; Peart, Edwards and Donaldson, 1972). One exception to this rule is the dairy-type, cross-bred ewe which exhibits a sustained lactation pattern i.e. high milk yield is maintained over a considerably longer period compared with the traditional mutton breed ewe (Peart et al., 1979).

Level and pattern of milk synthesis and secretion are under endocrine control (Fulkerson, 1979; Cowie, Forsyth and Hart, 1980). However, the physiological processes involved in the metabolism of nutrients and milk precursors and the endocrine control of these processes during lactation are complicated and poorly understood. A more detailed knowledge of the hormonal control of milk synthesis and secretion under a range of nutritional circumstances and with different ewe genotypes could facilitate more precise control of the level of milk production under a wide range of field conditions and perhaps the stimulation of sustained high yields by artificial means, such as hormone therapy techniques.

In the past, studies have been confined mainly to the role of individual hormones, examined without reference to associated changes in other hormones or nutritional status. As a consequence of this, at least in part, the data are often apparently contradictory and confusing. A more integrated approach was adopted by Hart and co-workers (Cowie et al., 1980) in studies involving dairy cows, with the result that some key endocrine factors were identified and relationships between hormones, and between hormones and nutrient status were characterised and related to the level of milk production. However, this work was confined to a study of effects of cow genotype on milk production and associated endocrine status. Little is known of the effects on overall endocrine status of either cattle or sheep of other parameters, such as suckling stimulus and nutrition, which are known to influence milk yield.

The work reported herein was designed to determine the circulating levels, during lactation, of a number of hormones known to be involved in the control of milk production and nutrient metabolism in the ewe. In particular, the aim was to describe changes in the relationships between hormone levels throughout lactation. Differences in suckling stimulus, ewe nutrition and ewe genotype were used to manipulate milk production levels. It was intended that the use of three different factors known to affect milk yield would make it possible to determine whether or not the same hormone factors were involved in the control of milk production in each case.

In addition to the anticipated effects of litter size, nutrition and genotype on milk yield, time of lambing was also examined as part of the litter size study in order to observe possible seasonal effects on the mechanism of control of milk production, particularly as some of the hormones involved in the control of milk production are known to exhibit seasonal variation (Trenkle, 1978).

CHAPTER 2

REVIEW OF LITERATURE

I. FACTORS AFFECTING MILK PRODUCTION IN THE EWE

INTRODUCTION

The suckling lamb is entirely dependant on maternal milk production, as a source of nutrients, during the first 3 to 4 weeks of life and during this period lamb growth rate is highly correlated with the amount of milk consumed (Wallace, 1948; Burris and Baugus, 1955). Indeed lamb growth rate has been used as an estimate of maternal milk production in a number of studies (Owen, 1957; Doney and Munro, 1962; Peart, 1967). Subsequently, as milk yield falls and consumption of solid food increases, the lamb becomes progressively less dependant on milk although lamb growth is still related to milk intake during mid- and late-lactation (Wallace, 1948; Burris and Baugus, 1955; Doney, Peart, Smith and Sim, 1983) and increases in herbage intake do not compensate for falling milk intakes during this period (Gibb, Treacher and Shanmugalingam, 1981; Doney et al., 1983).

Initially milk production of the suckled ewe is in excess of the lambs' ability to extract milk. As the demand of the lambs increases milk production responds up to a peak level which generally occurs between week 2 and 6 of lactation. Proportionally about 0.70 of the total yield in a single lactation is produced during the first 5 to 6 weeks of lactation (Peart, 1967; Peart, 1968a). Beyond the peak, milk production tends to decline steadily until milk synthesis ceases or the lambs are weaned. When lambs are weaned before lactation has ended abrupt involution of the mammary tissue is induced by removal of the suckling stimulus (Cowie et al., 1980).

Water, lipids, proteins, carbohydrates and minerals are the major consitutuents of milk; the relative proportions of these vary with stage

of lactation. The composition of colostral milk, which is produced during the first 2 to 3 days of lactation and is essential for lamb survival, differs markedly from milk produced in later lactation in that it contains immunoglobulins (Cowie et al., 1980). Fat and protein contents together with energy value are higher and lactose content lower in the colostrum compared with milk produced during the subsequent lactation (Peart et al., 1972).

Milk fat, protein, and ash contents and energy value generally decline during early lactation and increase in late lactation, while lactose content exhibits the opposite pattern (Gardner and Hogue, 1964; Peart et al., 1972; Peart, Edwards and Donaldson, 1975).

The potential of the mammary gland for milk synthesis and secretion is determined ultimately by the number and biosynthetic activity of the mammary secretory cells. Variation in milk production is associated with both instrinisic factors (e.g. ewe genotype and body size) and external factors which may influence milk yield (e.g. suckling stimulus and nutrition).

In the following section factors affecting milk production, pattern of milk production and milk composition in the ewe are discussed.

GROWTH AND DEVELOPMENT OF THE MAMMARY GLAND DURING PREGNANCY

Foetal influence

Mammary development during pregnancy is under endocrine control (Cowie et al., 1980). Foetal number affects both hormone status and mammary gland growth during pregnancy. Rattray, Garrett, East and Hinman (1974) found that in late pregnancy polytocous ewes had heavier mammary glands than monotocous ewes, together with mammary gland compositional differences. This was considered to be indicative of a

higher amount of secretory tissue and secretory material in the heavier glands.

Placental lactogen, a hormone secreted by the placenta, may be involved in the control of mammary gland growth and development (Fulkerson 1979; Cowie et al., 1980), and an increase in circulating placental lactogen concentration has been associated with increments in foetal number in the ewe (Martal and Djiane, 1977a; Taylor, Jenkin, Robinson, Thorburn, Friesen and Chan, 1980; Butler, Fullencamp, Capiello and Handwerger, 1981) and in the goat (Hayden, Thomas, Smith and Forsyth, 1980). Furthermore, Hayden, Thomas and Forsyth (1979) demonstrated that foetal number was in turn positively correlated with the weight of lobulo-alveolar tissue in the mammary gland.

Although the evidence is circumstantial, it does suggest that growth and development of the mammary gland during pregnancy may be influenced by the number and perhaps size of the lambs in utero.

Nutritional influence

A reduction in the size of the mammary gland at parturition has been observed in ewes which were undernourished during pregnancy (Wallace, 1948; Thompson and Thompson, 1948-9; Mellor and Murray, 1985), although the degree of nutrient restriction in all these cases was fairly severe. There is no information on qualitative changes which take place within the mammary glands of ruminants under conditions of inadequate nutrient supply but Knight and Peaker (1982a) have observed a reduction in the size of the secretory cell population in mouse mammary glands following a short period of fasting. They suggest that this response may be attributable either to a direct effect of nutrient insufficiency on mammary gland development or to an indirect endocrine effect. It has been suggested that the effect of high levels of nutrition on reducing mammary gland growth and development in heifers (Sejrsen,

Huber, Tucker and Akers, 1982) is related to decreased circulating GH concentration (Sejrsen, Huber and Tucker, 1983).

EFFECT OF NUMBER OF LAMBS BORN

In the ewe, evidence relating the number of lambs born to subsequent milk yield is both limited and conflicting. Peart et al., (1972) noted an increase in the mean daily colostrum yield of ewes which bore twins and reared singles compared with ewes which bore and reared single lambs and also ewes which bore triplets and reared twins compared with ewes which bore and reared twin lambs. However, there is no information on how long this effect persisted. Barnicoat, Logan and Grant (1949), Alexander and Davis (1959) and Doney and Munro (1962) observed no difference in mean milk yield over the whole lactation of single-suckled ewes which had borne either single or twin lambs. In contrast, fairly large differences in milk production (measured for 50 days after removal of the kids) were observed in hand-milked goats which had borne triplets (0.48 proportional increase in yield) and twin (0.28 proportional increase in yield) kids compared with goats which had borne single kids (Hayden et al. 1979).

In short, there is some indication from the literature that the number of lambs born may have some influence on subsequent milk production levels of their dams. This effect may be of short duration and easily masked by other factors directly influencing milk production during the subsequent lactation period.

EFFECT OF SUCKLING STIMULUS

Number of lambs suckled

Milk Production

Many studies have shown that ewes suckling twin lambs produce

more milk than those suckling singles, irrespective of the number born (Davis, 1963; Alexander and Davis, 1959; Doney and Munro, 1962; Peart et al., 1972; Peart et al., 1979; Doney et al., 1981). Proportionally, increases range from almost none to over 0.70 (Treacher, 1983) and an average proportional increase of 0.40 has been calculated from results of numerous studies (Treacher, 1978). Further increases in milk production have been shown in triplet or quadruplet-suckled ewes (Kornerev, 1974; Peart et al., 1972; Peart, Edwards and Donaldson, 1975; Loerch, McClure and Parker 1985). In the studies of Peart et al. (1972) and Peart, Edwards and Donaldson (1975) proportional increases in total milk yield measured over the first 12 weeks of lactation were between 0.40 and 0.63 in multi-suckled compared with single-suckled ewes. Stimulation of milk production in response to increased suckling stimulus only occurs during the early stages of lactation. Milk yields of single- and multi-suckled ewes do not generally differ significantly after peak milk production (Peart et al., 1972).

Pattern of lactation

In general, milk production of multi-suckled ewes increases at a greater rate, reaches a higher peak, often at an earlier stage of lactation, and declines at a higher rate compared with single-suckled ewes (Peart et al., 1972; Louda and Doney, 1976).

Milk composition

Differences in overall milk constituent content are generally independent of the number of lambs suckled (Slen, Clark and Hironaka, 1963; Peart, et al., 1979; Doney et al., 1981). However in some studies higher milk fat content and energy values of milk have been recorded in multi- compared with single-suckled ewes (Gardner and Hogue, 1964; Peart et al., 1972; Peart, Edwards and Donaldson, 1975).

Effect of lamb genotype

Milk production

A number of studies (using cross-fostering techniques) have indicated that the genotype of the suckling lamb may influence maternal milk production (Moore, 1966; Langlands, 1972; Doney et al., 1981). Proportionally differences with lamb genotype in total milk production over the first 9 to 12 weeks of lactation amount to 0.20 in single-suckled ewes (Langlands, 1972; Doney et al., 1981) and between 0.04 and 0.10 in twin-suckled ewes. The effect has been shown to persist during the first 5 to 8 weeks of lactation (Moore, 1966; Doney et al., 1981).

It has been suggested that the effect is related at least in part, to differences in the appetite of the lambs which in turn may be a reflection of their growth potential. Langlands (1972) observed a positive relationship between lamb growth rate and voluntary food intake. Other comparative studies have demonstrated that lamb genotypes normally considered to possess a higher growth potential, for example cross-bred lambs, are able to stimulate their dams to produce a greater quantity of milk compared with lamb genotypes of lower growth potential. (Peart, Doney and MacDonald, 1975; Doney et al., 1981).

Pattern of lactation

The effect on the lactation curve of differences in lamb demand with lamb genotype is similar to that observed with the increase in number or lambs suckled (Doney et al., 1981).

Milk composition

Doney et al. (1981) reported a higher overall milk fat content associated with an increase in milk yield, in ewes suckling Suffolk, cross-bred lambs compared with Scottish Blackface (SBF) lambs. There are, however, very few data with which to make any comparisons. Langlands (1972) observed non-consistent differences in milk fat content associated

with differences in lamb genotype despite clear differences in maternal milk production. There is no information on production of any other milk constituents in relation to differences in milk production associated with genotype of lamb suckled.

EFFECT OF EWE GENOTYPE

Milk Production

The level and pattern of milk production is known to be influenced by the breed of the ewe (Flamant and Casu, 1978; Peart, 1982). In addition to breed differences there are variations in milk yield within breeds attributable to differences in the milk production potential of individual animals. Barnicoat et al. (1949), using New Zealand Romney ewes, observed a three-fold difference in milk production between the highest and lowest yielder within a single group of ewes, managed under similar nutritional and environmental conditions.

In the case of dairy breeds, where it is possible to obtain accurate measures of milk production, differences in milk yield potential associated with ewe genotype are obvious. Estimation of milk production in the suckled ewes is less satisfactory and reflects the interaction between the potential of the ewe to produce milk and the potential of the lamb to extract milk. In general it can be said that estimated milk production of single-rearing ewes during early lactation is a reflection of the lamb's demand which is more likely to be influenced by differences in lamb genotype (Doney et al., 1981), while milk production of twin-rearing ewes probably provides a more accurate estimate of the milk production potential of the ewe (Slen et al., 1963). The most valid ewe breed comparisons have been obtained from studies where the suckling stimulus has been equalised for all maternal breeds by cross-fostering

lambs at birth. For example, over the first 9 weeks of lactation, Border Leicester (BL) ewes produced proportionally 0.54 more milk compared with Merino ewes when both breeds were rearing BL lambs (Langlands, 1972). Similarly a significantly higher yield was observed when East Friesland x Scottish Blackface ewes were constrasted with pure Blackface ewes when all ewes were rearing Suffolk cross-bred lambs (Doney et al., 1981).

Pattern of lactation

Profiles of milk production over the whole lactation can vary quite markedly with ewe genotype. The Merino breed exhibits a much flatter milk curve than British breeds (Davis, 1963), while levels of milk production in dairy ewes tends to remain elevated for a much longer period compared with non-dairy breeds (Louda and Doney, 1976).

Milk composition

Information concerning differences in milk composition with ewe genotype is sparse. Whilst concentrations of other milk constituents appear to be relatively consistent between breeds, both positive and negative relationships between milk fat content and genotypically induced differences in milk production have been recorded (Gardner and Hogue 1966; Ricordeau and Flamant, 1969; Doney et al., 1981).

EFFECT OF EWE AGE AND PARITY

The effect of age and parity on milk production has been reviewed by Treacher (1978 ; 1983). From the limited information available for suckling ewes it is generally agreed that milk production reaches a maximum level between lactation 3 and 6, and the rate of decline with each subsequent lactation depends on the current level of nutrition and management.

EFFECTS OF EWE BODY SIZE AND WEIGHT

There is an important distinction between body size, which is a reflection of skeletal size, and body weight, which is related to both skeletal size and body composition of the animal. Many studies have examined the relationship between milk yield and either body weight or size. While results are often conflicting (Treacher, 1978) it is generally assumed that ewes of larger body size produce more milk than those of smaller size.

EFFECT OF NUTRITION

During pregnancy

Undernutrition during pregnancy can delay the onset of lactation (Treacher, 1970; McCance and Alexander, 1959) and reduce subsequent milk yield (Wallace, 1948; Treacher, 1970). The effect on milk production, however, is highly dependant on the severity of undernutrition during pregnancy and the level of nutrition during lactation. For example, Peart (1967) and Maxwell, Doney, Milne, Peart, Russel, Sibbald and MacDonald (1979) observed only a slight reduction in milk production in ewes which were undernourished during pregnancy and fed ad libitum during lactation.

Specific effects of nutrition during pregnancy on the growth and development of the mammary gland have been discussed earlier. In addition to this, nutrition affects the growth and development of foetus during pregnancy and this is turn affects the demand of the lamb for milk in early lactation.

The growth of the ovine conceptus is highly sensitive to changes in maternal nutrition during pregnancy (Mellor, 1983), and undernutrition during pregnancy induces lower lamb birth weights (Alexander, 1974; Russel, Doney and Reid, 1967). The extent of the reduction of lamb

birth weight depends on the severity of undernutrition which is imposed during pregnancy (Russel et al., 1967). While subsequent milk production may be influenced indirectly as a result of reduced intake by smaller lambs (Peart, 1967), it is not known to what extent birth weight must be reduced before such an effect on maternal milk production is produced. Peart (1982), suggested that a reduction in milk production would be unlikely, unless lamb birth weight was reduced by more than a factor of 0.25.

Nutrition during pregnancy may also produce substantial differences in ewe live weight and body condition at parturition (Gibb and Treacher, 1982) although the effect of this on subsequent milk yield depends almost entirely on the level of nutrition during lactation (Peart, 1967).

During lactation

The nutritional requirements of the ewe are highest during lactation and in some cases may be proportionally up to 0.70 higher compared to requirements during late pregnancy (Agricultural Research Council, 1980). In early lactation, despite a substantial increase in voluntary food intake, dietary nutrients are not always sufficient to meet the requirements of high-yielding ewes (Robinson, 1973). Therefore milk production is often accompanied by substantial losses in ewe body condition and live weight.

Effects on milk production of nutrition during lactation are generally much greater than the indirect effects of nutrition during pregnancy. The size of the reduction in yield induced by nutrient restriction depends on the severity, duration and timing of the undernutrition imposed. For example, feed restriction during the first week of lactation produces very little effect on milk production, although continued restriction for the following 3 weeks produces a highly significant reduction in yield (Jagusch, Jay and Clark, 1972). Milk production is restored to the level of unrestricted ewes following

removal of feed restriction (Jagusch et al., 1972). Stimulation of milk production by increasing nutrient supply on the other hand is effective only during early lactation (Peart, 1970b).

Voluntary food intake

Reports of voluntary food intake patterns in lactating ewes have been reviewed by Foot and Tissier (1978). The initiation of lactation in ewes leads to hypertrophy of the alimentary tract (Fell, Campbell, Mackie and Weekes, 1972). Voluntary food intake increases during lactation, generally reaching a peak between 6 to 9 weeks and then steadily declines (Meat and Livestock Commission, 1981 ; Doney et al., 1983). The main factors affecting the level and pattern of voluntary food intake during lactation include diet type, prepartum nutrition, suckling intensity and ewe genotype (Foot and Tissier, 1978).

Under conditions of high nutrient requirements, the level of food intake depends on the rate of passage of food within the digestive system. Therefore ewe nutrient intake is reduced by poor quality diets of low digestibility (Hadjiperis and Holmes, 1966; Foot and Tissier, 1978). In ewes fed a high quality feed voluntary food intake is often high (Peart, 1967) and may be in excess of requirements for maintenance and production (Corbett, 1968; Peart et al., 1972).

Pre-partum nutrition may influence voluntary food intake during the subsequent lactation and voluntary food intake has been shown to be negatively associated with body condition at parturition (Peart, 1970b). Cowan, Robinson, MacDonald and Smart (1980) observed a similar effect, the size of which was influenced by the energy content of the diet fed during lactation. Poor nutrition during pregnancy may be compensated by higher voluntary food intakes during lactation (Foot and Tissier, 1978) although Treacher (1970; 1971) observed non-significant differences in intake of groups of ewes, differentially fed during pregnancy.

The voluntary food intake of ewes suckling twin lambs (like their milk yield) generally exceeds that of ewes suckling singles (Foot and Tissier, 1978) although some studies have indicated that voluntary food intake of single- and twin-rearing ewes does not differ significantly, despite significant differences in milk production (Doney, Peart, Smith and Louda, 1979 ; Maxwell et al., 1979; Doney et al., 1983).

Other factors which influence voluntary food intake and may account for some of the individual variability between animals are ewe body weight and age (Foot and Tissier, 1978) and ewe genotype (Doney et al., 1983).

Energy and protein supply

Milk production varies with both the energy (Treacher, 1971; Gardner and Hogue, 1964; Cowan et al., 1980) and protein content of the diet (Calderton-Cortes, Robinson, McHattie and Fraser, 1977; Cowan, Robinson, McHattie and Pennie, 1981)

The efficiency of utilisation of energy for milk production is generally fairly constant, changing only slightly with metabolisability of the diet (ARC, 1980). Gardner and Hogue (1964) reported a greater energetic efficiency of milk production in twin-suckled ewes compared with single-suckled ewes, although this effect was not confirmed in a subsequent study (Gardner and Hogue, 1966).

Robinson (1978; 1980) has characterised patterns of milk production and body weight changes in response to different intakes of metabolisable energy and crude protein in the diet. Increasing the crude protein content of the diet above the metabolisable energy:crude protein ratio requirement for a given level of milk production, stimulates milk yield provided that the potential of the ewe has not been attained. This increase is sometimes accompanied by increased liveweight loss (Robinson, Fraser, Gill and McHattie, 1974; Calderton-Cortes et al.,

1977) although it may be associated with an increase in the efficiency of use of body tissue for milk production (Cowan, et al., 1981). The source of protein in the diet is also of considerable importance, and in particular the extent of its degradation in the rumen. Milk production increases in response to decreased rumen degradability of the protein source in the diet (Gonzalez, Robinson, McHattie and Fraser, 1982).

The contribution of body tissues to milk production depends very much on the interaction between nutrient intake and extent of body reserves during lactation. The rate of adipose tissue mobilisation increases with body fat content although the response tends to decline as metabolisable energy intake increases (Cowan, Robinson and McDonald, 1982). Thus, adipose tissue content is likely to be of greater importance with lower levels of metabolisable energy intake, although body reserves will not support milk production if intake during lactation is severely depleted (Peart, 1968). Furthermore, Cowan et al. (1980) observed an apparent decrease in the energetic efficiency of milk production when the contribution of adipose tissue for milk sythesis increased. When ewes are in negative energy and N balance, milk production may be limited by the amount of dietary protein as labile protein resources are scarce (Cowan, Robinson, Greenhalgh and McHattie, 1979; Cowan et al., 1980).

SUMMARY AND CONCLUSION

Milk production is influenced by many different factors. In addition to the direct effects of these factors, there may be many interactions between them which may confound experimental results and make interpretation more difficult.

The effects of each of the factors on milk production must be mediated through changes in endocrine status and the factors which in turn affect endocrine status.

II. THE ENDOCRINOLOGY OF LACTATION IN THE EWE

INTRODUCTION

In all mammalian species the lactation cycle can conveniently be divided into four stages :-

(i) Growth and development of the mammary gland (mammogenesis).

(ii) Transformation of mammary cells from a non-secretory to secretory state and the onset of milk secretion (lactogenesis).

(iii) Maintenance of milk synthesis and secretion.

(iv) Reduction of milk synthesis and regression of mammary tissue (involution).

Understanding of the endocrine control of these processes in ruminant and other species has been comprehensively reviewed (Convey, 1974; Fulkerson, 1979; Cowie et al., 1980; Delouis, Djiane, Houdebine, Terqui, 1980; Tucker, 1981 ; Knight and Peaker, 1982b; Forsyth, 1983).

The following section contains a brief review of endocrine factors implicated in the control of the first 3 stages of the lactation cycle in the ewe. In cases where there is little or no information available concerning sheep, reference is made to work using other species.

MAMMOGENESIS

Milk yield potential is ultimately determined by the number of secretory cells in the mammary gland. Prior to conception in the ewe the mammary gland consists almost entirely of adipose tissue interspersed with a rudimentary duct system and a small amount of lobulo-alveolar tissue, which develops and regresses in accordance with the hormonal changes associated with ovarian cycles (Fulkerson, 1979; Cowie et al., 1980). During pregnancy there is a marked increase in the size and complexity of the duct system and much cell proliferation and

differentiation takes place forming lobulo-alveolar structures containing the specialised secretory cells (Knight and Peaker, 1982b). Mammogenesis, as estimated by the concentration of mammary deoxyribonucleic acid (DNA) which is an index of cell number, is complete at parturition (Anderson, 1975).

Endocrine changes during pregnancy, when mammary tissue is developing, include a gradual increase in progesterone concentration. Levels rise sharply at approximately day 50 of pregnancy, when placental production of progesterone begins, and reach a maximum level between days 120 and 140 of pregnancy at a time when mammary secretory tissue development is maximum (Bassett, Oxborrow, Smith and Thorburn 1969; Cowie et al., 1980). There is a more gradual increase in oestrogen concentration throughout pregnancy (Cowie et al., 1980) and also an increase in the concentration of placental lactogen between days 40 and 60 of pregnancy which reaches a maximum level during the final weeks of pregnancy (Kelly, Robertson, Friesen, 1974; Handwerger, Crenshaw, Maurer, Barrett, Hurley, Golander and Fellows, 1977; Cowie et al., 1980), again during the period of maximum mammary secretory tissue development. Circulating prolactin (Kelly et al., 1974) and growth hormone (GH) concentrations remain fairly low during early pregnancy, but tend to increase during late pregnancy (Blom, Hove, Nedkvitne, 1976; Vernon, Clegg and Flint, 1981a). Adrenal corticosteroid concentration decreases throughout pregnancy (Cowie et al., 1980).

Mammary gland growth is probably primarily under the control of the ovarian steroids (i.e. oestrogen and progesterone) and anterior pituitary hormones (i.e. prolactin and GH) (Fulkerson, 1979; Cowie et al., 1980; Forsyth, 1983), although in some species, for example sheep, goats and rats, hormones of feto-placental origin (in particular placental lactogen), are thought to be directly involved (Cowie et al., 1980).

Other hormones implicated in the control of mammary growth and development include adrenal corticosteroids, insulin, relaxin and the thyroid hormones (Fulkerson, 1979; Cowie et al., 1980; Tucker, 1981, Forsyth, 1983).

The involvement of these hormones has been demonstrated experimentally and lobulo-alveolar development has been achieved in vitro using mammary explants from ewes incubated with a combination of hormones including the ovarian steriods, adrenal corticosteroids, prolactin and GH (Jeulin-Bailly, Delouis and Denamur, 1973; Fulkerson, 1979; Cowie et al., 1980). Furthermore the effectivness of treatment of non-pregnant ewes with combinations of ovarian steriods, adrenal corticosteroids and prolactin in induction of development of lobulo-alveolar structures is well documented (Fulkerson and McDowell, 1974; Head, Delouis, Terqui, Kann, Djiane, 1980; Schams, Rüsse, Schallenberger, Prokopp and Chan, 1984).

Some controversy remains in the literature regarding the role of the anterior pitutary hormones in the control of mammary gland growth and development. Hooley, Campbell and Findlay (1978) observed that suppression of prolactin concentration using 2-bromo-α-ergocryptine during treatment of non-pregnant ewes with ovarian steriods resulted in reduced milk yield in the subsequent lactation compared with control animals, presumably as a result of reduced mammary growth. In contrast, Delouis et al. (1980) demonstrated that alveolus formation was not affected when ewes were treated with 2-bromo-α-ergocryptine and mammary growth was not significantly reduced during a normal pregnancy in hypophysectomised animals where the supply of anterior pituitary hormones (i.e. prolactin and GH) was completely abolished (Denamur and Martinet, 1961).

It has been suggested that hormones of placental origin (in particular placental lactogen) may partially or wholly take over the function of the anterior pituitary gland in relation to mammary growth in the absence of anterior pituitary hormone support (Hooley et al., 1978; Schams et al., 1984). The close association between circulating levels of placental lactogen and the phase of rapid morphological and secretory mammary tissue development during the later stages of gestation in the ewe, provides circumstantial evidence for the involvement of this hormone. Purified placental lactogen possesses both prolactin-like and growth hormone-like properties (Martal and Djiane, 1977b), and has been found to exhibit mammogenic effects in vitro in an experiment using mammary explants from pregnant ewes (Djiane and Kann, 1975). It has been suggested that placental lactogen concentration is related to litter size in ewes (Butler et al., 1981) and subsequent milk production in goats (Hayden et al., 1979). It is possible that placental lactogen may exert some control over the extent of mammary gland growth in relation to litter size.

Summary

Mammogenesis is the ewe is essentially complete at parturition. Key hormones thought to be involved in the growth and development of the mammary gland during pregnancy are the ovarian steriods, anterior pituitary hormones and/or hormones of feto-placental origin, in particular placental lactogen

LACTOGENESIS

The onset of copious milk production generally coincides with parturition in the ewe, although the transition of mammary epithelial cells from a non-secretory to secretory state occurs prior to parturition. Extraction of small amounts of secretory material from the mammary gland has been achieved as early as 30 days prepartum in the ewe

(Hartmann, Trevethan and Shelton, 1973). Denamur (1965) detected lactose synthesis by day 90 of pregnancy. During late pregnancy and around the time of parturition, progesterone and placental lactogen concentrations are generally decreasing while oestrogen, prolactin, adrenal corticosteroid, prostaglandin F2α, oxytoxin and GH concentrations are increasing (Lamming, Moseley and McNeilly, 1972; Thornburn, Nicol, Bassett, Scutt and Cox, 1972; Fulkerson, 1979; Vernon et al., 1981a).

The hormones thought to be primarily involved in the differentation and increased biosynthetic activity of mammary epithelial cells during late pregnancy include prolactin and adrenal corticosteroids (Fulkerson, 1979; Cowie, et al., 1980). It has been demonstrated, using in vitro mammary explants, that combinations of prolactin, adrenal corticosteroids and insulin are required for the specific changes in mammany nucleic acid synthesis, rates of synthesis of key mammary biosynthetic enzymes and changes in mammary gland metabolism required for lactogenesis (Baldwin and Louis, 1975; Fulkerson, 1979; Cowie et al., 1980; Houdebine, Djiane, Dunsanter-Fourt, Martel, Kelly, Devinoy and Servely, 1985). Furthermore the same hormones are also thought to stimulate the growth of myoepithelial cells and their response to oxytocin (Fulkerson, 1979), which is essential for the process of milk removal at suckling or milking.

It has been postulated that in several species, including the ewe, lactogenesis is ultimately controlled by the withdrawal of circulating progesterone (Kuhn, 1983). Although an initial increase in the lactose content of the mammary gland was observed as early as 30 days prepartum (Hartmann et al., 1973), any further increase in lactose content of mammary tissue was observed only following a decline in progesterone concentration (Hartmann et al., 1973). Studies, in vitro, have demonstrated the inhibitory influence of progesterone on prolactin and adrenal corticosteroid induced milk synthesis (Baldwin and Louis,

1975 ; Fulkerson, 1979). It appears, therefore, that mammary epithelial cells gain the ability to synthesise milk constituents such as lactose at least a month prior to parturition but are prevented from synthesising significant amounts of milk constituents by the presence of progesterone.

Notwithstanding the substantial evidence relating onset of lactation to withdrawal of circulating progesterone concentration it is evident that an increase in the concentration of 'lactogenic hormones' is also important in the control of lactogenesis. Indeed, Delouis and Denamur (1967) demonstrated that the inhibitory effect of progesterone on lactogenesis could be overcome by pharmological doses of 'lactogenic hormones' after day 95 of pregnancy. In another experiment suppression of prolactin concentration using 2-bromo-α-ergocryptine immediately prior to parturition resulted in delayed onset on milk production and reduced milk yields (Kann, 1976). However, artificial induction of lactation was not inhibited by suppressed prolactin concentration in the presence of raised levels of adrenal corticosteroids (Fulkerson, McDowell and Fell, 1975 ; Hooley et al., 1978). It has been suggested that the effects of some of the hormones involved in lactogenesis may be mediated through changes in the sensitivity of the mammary gland to these hormones (Fulkerson, 1979).

Other hormones which also elicit a lactogenic response, include oxytocin and prostaglandin F2α (Fulkerson, 1979), although in view of evidence relating increases in concentrations of these hormones to increases in prolactin and possibly adrenal corticosteroid and/or GH concentrations (Fulkerson, 1979), it is unlikely that these hormones have a direct effect on lactogenesis.

Recent evidence has suggested that the mammary gland may be capable of synthesising a hormone or hormones which can inhibit milk synthesis until removal by suckling or milking. Mammary epithelial cell

differentiation in dairy cows was accelerated when one side of the mammary gland was milked prior to parturition compared with the other side which was not milked until after parturition (Akers, Heald, Bibb and McGilliard, 1977). Hormones implicated in this control mechanism are prostaglandin F2α, oestradiol 17-β and progesterone (Maule Walker, 1984).

Summary

Key hormones involved in the initiation of milk synthesis and secretion are prolactin and adrenal corticosteroids. Withdrawal of progesterone at parturition may have a permissive influence on the action of these hormones. The mammary gland may produce hormones which inhibit copious milk synthesis until suckling or milking commences.

MAINTENANCE OF LACTATION

A variety of approaches has been used to investigate the hormones involved in the maintenance of milk production, such as hormone infusion, hormone inhibition and examination of hormone levels in relation to level of milk production and stage of lactation (Fulkerson, 1979; Cowie et al., 1980).

Hormones essential for restoration of milk yield following hypophysectomy in the ewe are prolactin, GH, thyroxine (T^4) and adrenal corticosteriods (Denamur, 1971). Insulin is also required to maintain mammary secretory cell viability in vitro (Baldwin and Louis, 1975; Cowie et al., 1980), indicating that all these hormones are likely to be involved in the control of milk production.

Prolactin

Prolactin is undoubtely essential for maintenance of milk production in several non-ruminant species (Cowie et al., 1980 ; Forsyth, 1983). Prolactin may also have an integral role in the control of milk production in ruminant species. Circumstantial evidence for this is the release of prolactin at suckling (McNeilly, Moseley and Lamming, 1972)

and the decrease in basal prolactin levels and the prolactin response to suckling as lactation proceeds (McNeilly, 1971; McNeilly et al., 1972), which has been shown to parallel changes in milk production (Koprowski and Tucker, 1973a). In general, however, stimulation or suppression of prolactin levels does not influence milk production in ruminant species, although results vary both within and between species.

In lactating ewes, suppression of prolactin levels, using 2-bromo-α-ergocryptine, resulted in proportional reductions in milk yield ranging from 0.20 - 0.70 (Kann, 1976; Hooley et al., 1978). The fact that this reduction in milk production could be overcome by simultaneous infusion with exogenous prolactin (Hooley et al., 1978) suggests that prolactin does have a role in the control of maintenance of milk production in the ewe.

Stimulation of circulating prolactin levels has produced varied results. An increase in milk yield following administration of various drugs known to stimulate prolactin release was obtained by Bass, Shani, Givant, Yagil and Sulman (1974). Treatment of ewes with exogenous prolactin, however, failed to produce any response in terms of milk production (Denamur and Martinet, 1970; Morag, Shani, Sulman and Yagil, 1971).

It is possible that maintenance of lactation depends on a threshold level of prolactin below which prolactin levels are only sometimes suppressed by 2-bromo-α-ergocryptine. Furthermore the threshold level may vary according to species and even within ruminant species. This would explain, at least in part, the reduction in milk production when prolactin levels were suppressed and the absence of any increase in milk production when prolactin levels were stimulated.

It is known that prolactin is involved in the transcription process at the cellular level (Baldwin and Louis, 1975; Fulkerson, 1979; Collier

McNamara, Wallace and Dehoff, 1984). Since milk yield is not necessarily affected to any great extent by treatment with 2-bromo-α-ergocryptine (Cowie et al., 1980) it must be assumed that this process can continue in the presence of low levels of prolactin.

It is uncertain whether or not prolactin has a role in the control of peripheral metabolism in ruminant species. In rats there is evidence that prolactin affects lipid synthesis in adipose tissue during lactation (Bauman and Currie, 1980; Bauman and Elliott, 1983; Vernon and Flint, 1984). No evidence of a direct influence of prolactin on adipose tissue metabolism has been recorded in sheep (Manns and Boda, 1965; Vernon, Clegg and Flint, 1986). Another postulated role for prolactin during lactation is stimulation of gut hypertrophy (Mainoya, 1978), but there is little or no evidence of this in ruminant species.

In summary, the evidence to date suggests that within the normal range of circulating levels, prolactin is not of primary importance in the control of variation in milk production in the ewe but if levels are markedly suppressed, milk production may be reduced.

Growth hormone

Growth hormone is released at milking in ewes, although this is somewhat variable (Martal, 1975). Results from work in goats suggest that unlike prolactin, GH may not be released simply in response to tactile stimulation of the udder (Hart and Linzell, 1977); release may be related to changes in physiological state associated with suckling.

GH levels are higher in high- compared with low-yielding dairy cattle (Hart, Bines, Balch and Cowie, 1975; Hart, Bines, Morant and Ridley, 1978), and changes in GH concentration are positively correlated with changes in milk production and negatively correlated with changes in live weight throughout lactation (Hart, Bines and Morant, 1979).

Treatment of lactating ewes with exogenous GH or exogenous GH

releasing factors at various stages of lactation has produced a significant increase in milk yield (Jordan and Schaffhausen, 1954; Denamur and Martinet, 1970; McDowell and Hart, 1983; Hart, Chadwick, James and Simmonds, 1985). To date these studies have been confined to short-term treatment with a GH preparation. In the lactating dairy cow, however, increases in milk yield have also been demonstrated in cows treated on a long-term basis (Bauman and McCutcheon, 1986).

Although evidence for the involvement of GH in the control of milk production is overwhelming in ruminant species (Fulkerson, 1979; Cowie et al., 1980; Bines and Hart, 1982; Bauman and McCutcheon, 1986) its mode of action is poorly understood. However it is clear that there is an increase in the gross efficiency of milk production following treatment with GH, which is not thought to be related to any change in the digestive processes or energy costs associated with maintenance and milk production (Peel, Bauman, Gorewit and Sniffen, 1981; Tyrell, Brown, Reynolds, Haaland, Peel, Bauman and Steinhour, 1982).

It seems likely that GH stimulates milk production by virtue of an influence on the direction in which nutrients are partitioned within the body (Bines and Hart, 1977; Bines and Hart, 1978; Bauman and Currie, 1980; Hart, 1983; Bauman and Elliot, 1983; Thilsted, 1985). There is an abundance of evidence in the literature concerning the short-term (or acute) effects of GH treatment on metabolic processes. Both 'lipolytic' (adipose tissue mobilising) and 'diabetogenic' (glucose stimulating) proporties of GH have been demonstrated in sheep (Manns and Boda, 1965; Bassett and Wallace, 1966; Wallace and Bassett, 1966). However, up until now there has always been doubt about the heterogenity and degree of purification of GH preparations used (Hart, 1981; Bauman and McCutcheon, 1986). Recent evidence has shown that highly purified GH preparations do not produce such effects (Bauman and McCutchean, 1986)

and it has been postulated that acute effects of GH are attributable, at least in part, to contaminants in the preparations. Furthermore, when highly purified GH preparations are administered to lactating animals, the rise in milk production does not occur immediately but gradually over the next few days (Hart et al., 1985).

It has therefore been suggested that effects of GH on nutrient partitioning are more long-term (or chronic). The mechanisms by which this is achieved are not known but it has been suggested that GH may alter the responsiveness of certain tissues in order to facilitate the supply of key nutrients to the mammary gland and away from peripheral utilisation. The recently demonstrated antagonistic effect of GH on insulin action (Vernon, 1982; Hart, 1983) is one possible means by which GH could mediate these effects. Irrespective of the mode of GH action, it is not known what signals trigger GH release although decreases in the circulating concentrations of either glucose or non-esterified fatty acids (NEFA) have been suggested (Hertelendy and Kipnis, 1973; McDowell, 1983).

In addition to the action of GH on partitioning of nutrients in peripheral tissues, GH may have a direct effect at the level of the mammary gland itself. An increase in the rate of mammary blood flow has been demonstrated following GH treatment in lactating goats (Mepham, Lawrence, Peters and Hart, 1984); this may be a mechanism by which substrate supply to the mammary gland is increased. It is not known, however, whether the effect on mammary blood flow is caused by the action of GH or is increased as a result of increased metabolic activity within the mammary gland. Certainly results in ewes suggest that GH does not have a local action at the mammary gland (McDowell and Hart, 1983; McDowell and Hart, 1984).

In summary, GH has a key role in the control of milk production in the ewe. Factors which stimulate GH and the mode of action of GH during lactation are unclear.

Adrenal corticosteriods

Adrenal corticosteriods are released at milking in cattle (Koprowski and Tucker, 1973b). Results of several studies in cattle have produced both positive and negative relationships between circulating adrenal coricosteriod levels and stage of lactation (Cowie et al., 1980). There is also disagreement with regard to the effects of administration of exogenous adrenal corticosteriods on milk yield in lactating ruminants (Fulkerson, 1979), although treatment of ewes with hydrocortisone did not influence level of milk production (Denamur and Martinet, 1970).

In addition to the function of adrenal corticosteriods in the initiation (Fulkerson, 1979; Cowie et al., 1980; Forsyth, 1983) and possibly in the continuation of mammary secretory activity, corticosteriods are also involved in control of peripheral metabolic processes. Plasma cortisol is sensitive to changes in glucose status and when blood glucose levels are reduced, cortisol stimulates protein catabolism and gluconeogenesis from amino acids (Reilly and Black, 1973; McDowell, 1983) and may also reduce milk output (Baird, 1981). Although adrenal corticosteroids are not potent short-term regulators of glucose metabolism in ruminants (Bassett, 1978), it is possible that they influence energy metabolism in the long-term (Trenkle, 1981). Indeed it has been suggested that in fasted sheep adrenal corticosteroids may enhance the lipolytic effects of GH (unpublished data, cited in Trenkle, 1981).

The evidence suggests that circulating adrenal corticosteroid concentration is not a limiting factor in the control of milk production

although the role of adrenal corticosteroids in the partitioning of nutrients, particularly under conditions of energy deficit, requires further study.

Thyroid hormones

There is doubt about the relationship between thyroid hormone concentration and level of milk production. Pipes, Bauman, Brooks, Comfort and Turner (1963) observed an increased thyroid hormone secretion rate in high- compared with low-yielding dairy cows whereas Hart et al., (1978) showed no such difference. In general tri-iodothyronine (T^3) and T^4 concentrations are negatively correlated with milk production (Vanjonack and Johnson, 1975; Hart et al., 1979, Walsh, Vesley and Mahadevans, 1980) and circulating T^4 concentrations are lower in lactating compared with non-lactating cattle (Cowie et al., 1980).

In contrast, stimulation of milk production has been achieved by treatment of lactating cows with exogenous thyroid hormone preparations or thyro-active compounds (Cowie et al. 1980). The response is highly variable with increases ranging between 0.10 to 0.50 proportionally (Bartlett, Burt, Folley and Rowland, 1954; Fulkerson, 1979), and is dependent on body size, age and stage of lactation (Meites, 1961). Furthermore when treatment ceases or alternatively is prolonged there is a compensatory decrease in milk production (Meites, 1961; Cowie et al., 1980). It has been shown that thyroid hormone treatment is associated with an increase in metabolic rate coupled with an increase in food intake (Blaxter, Reineke, Crampton and Petersen, 1949) and consequently a decrease in the overall efficiency of milk production (Thomas and Moore, 1953).

Thyroid hormones are responsible for controlling metabolic rate and so have numerous effects on energy and protein metabolism (Bernal and

Refetoff, 1977). Knowledge of specific effects of thyroid hormone action is limited, although there is some evidence that the thyroid hormones are involved in control of glucose homeostatsis (McDowell, 1983). The mode of action of the thyroid hormones during lactation may be to selectively reduce the rate of metabolism in peripheral tissue and, as a consequence, to increase the supply of nutrients to the mammary gland (Collier et al., 1984).

In summary the thyroid hormones may be involved in the control of milk production by virtue of their effects on peripheral body metabolism.

Insulin

Insulin is required for maintenance of mammary tissue in vitro (Forsyth, 1971) and may be necesary for the uptake and utilisation of substrates at the mammary gland (Baldwin and Louis, 1975). However, in ruminants circulating insulin levels are generally negatively related to milk production (Koprowski and Tucker, 1973a; Hart et al., 1978) and positively associated with changes in body weight (Hart et al. 1978). Treatment of lactating animals with insulin generally results in reduced milk yields (Kronfield, Mayer, Robertson and Raggi, 1963).

Insulin together with glucagon exerts a strict control on circulating glucose levels (Bassett, 1975). In the non-lactating ruminant insulin stimulates the uptake and utilisation of glucose by peripheral tissues, inhibits gluconeogensis and glycogenolysis in the liver, stimulates uptake and incorporation of amino acids into protein, inhibits proteolysis, stimulates lipogenesis and inhibits lipolysis in adipose tissue (Bassett, 1975; 1978; Bauman, 1976; Brockman, 1978; McDowell, 1983). Glucagon stimulates gluconeogenesis and glycogenolysis in the liver and may stimulate lipolysis in adipose tissue (Bassett, 1978; Brockman, 1978; Bolton, Weekes, Godden and Armstrong, 1983). It follows, therefore, that insulin action is of great importance in relation to nutrient

availability for milk production as both glucose (Kronfield, 1976) and amino acid supply (Mepham, 1982) may be limiting factors in milk production.

It has been suggested that the action of insulin may be modified under physiological conditions such as lactation, so that glucose levels are maintained while nutrients are preferentially directed towards the mammary gland. Insulin levels are reduced in lactating compared with non-lactating cattle (Lomax, Baird, Mallison and Symonds, 1979) and sheep (Vernon et al., 1981) but in addition, reciprocal changes in insulin receptor numbers have been observed in adipose and mammary tissue during lactation, such that the capacity for fatty acid synthesis decreases in adipose tissue and increases in mammary tissue (Vernon et al., 1981; Vernon and Flint, 1984). Thus insulin levels and responses of peripheral tissues to insulin may be modified during lactation in order to accomodate increase nutrient requirements for milk production.

Summary

Prolactin and adrenal corticosteroids together with insulin are probably required for the the maintenance of synthetic activity in the mammary gland, while GH, thyroid hormones and insulin have effects on nutrient metabolism which influence the supply of milk precursors to the mammary gland. In addition, the adrenal corticosteroids and prolactin may also have a role in the control of nutrient metabolism during lactation.

Clearly the hormonal control of milk production is a complicated process involving many interacting factors. Work is required to characterise the relationships between hormones, and between hormone and milk production and associated nutrient metabolism.

III. SOME FACTORS AFFECTING CIRCULATING HORMONE CONCENTRATION

SUCKLING

Suckling or milking in ruminant species results in an increase in circulating concentrations of a number of hormones.

Prolactin

Circulating prolactin concentration is increased in response to suckling in the ewe (Fell, Beck, Brown, Katt, Cumming, Goding, 1972; Lamming, Mosely, McNeilly 1974). The response tends to decline as lactation proceeds and milk production decreases (McNeilly et al., 1972). Prolactin levels at suckling/milking have been found to be positively associated with the intensity of the suckling/milking stimulus (cow: Reinhart and Schams, 1974; goat: Hart and Linzell, 1977) and the duration of milking in the cow (Reinhardt and Schams, 1975).

Growth hormone

An increase in circulating GH concentration has been observed after milking in ewes (Martal 1975), although the response was not consistent. Hart and Linzell (1977), using goats, suggested that increased GH levels associated with suckling or milking may not be due entirely to tactile stimulation of the udder; circulating GH concentrations at milking were found to be unrelated to the number of teats milked indicating that the response was not related to the intensity of the suckling/milking stimulus.

Adrenal corticosteriods

Serum corticosteriod concentration was increased following milking in dairy cows (Smith, Convey, Edgerton, 1972; Koprowski and Tucker, 1973b). There is no information concerning the effect of suckling on circulating corticosteriod concentration in the ewe.

Thyroid hormones and Insulin

There is no evidence of increases in either circulating thyroid hormone or insulin levels in response to the suckling or milking stimulus in either ruminant or non-ruminant species.

PHOTOPERIOD/TEMPERATURE

Prolactin

Increases in circulating prolactin concentration under conditions of increased photoperiod and temperature are well documented in sheep (Pelletier, 1973; Forbes, Driver, El Shahat, Boaz and Scanes, 1975; Ravault, 1976; Forbes, Driver, Brown, Scanes and Hart, 1979).

GH, Adrenal corticosteroids, Thyroid hormones and Insulin

Changes in daylength do not affect GH (Forbes et al., 1979), adrenal corticosteroid (Chesworth and Easdon, 1983), thyroxine (Forbes et al., 1979) or insulin concentrations (Forbes et al, 1979; Forbes, 1982) in sheep.

Both increases and decreases in temperature generally stimulate GH and adrenal corticosteroid levels (Trenkle, 1978). Thyroxine levels are generally lower under conditions of increased temperature (Trenkle, 1978) although some results do not support this (Vanjonack and Johnson, 1975). There is little or no information concerning changes in insulin levels in response to changes in environmental temperature.

STRESS

Circulating concentrations of some hormones are increased in animals subjected to a variety of stressful stimuli including restraint, blood sampling, extremes of temperature and general disturbance (Trenkle, 1978). In particular, prolactin (Davis, 1972; Louw, Lishman and Botha, 1974) and adrenal corticosteriod levels (Chesworth and Easdon, 1983) are elevated by stressful stimuli although increases in GH have been observed in some cases (Trenkle, 1978). Bassett and Hinks

(1969) have shown that the size of the increase in adrenal corticosteriod levels is reduced in animals accustomed to procedures.

There is no evidence of an effect of stress on the thyroid hormones or insulin.

NUTRIENT SUPPLY

The effect of feeding on circulating hormone concentration depends on level of intake, diet composition, and the physiological status of the animal. While non-lactating animals have been studied extensively, little is known of lactating animals (Trenkle, 1978; Weekes and Godden, 1981).

Insulin

The biphasic release in insulin concentration following feeding is well documented in sheep (Bassett, 1974a & b; Trenkle 1978; Weekes and Godden, 1981) and insulin levels following feeding are positively related to intake and the amount of organic matter digested in the alimentary tract (Bassett, 1974a and b; Bassett, Weston and Hogue, 1971; Sejrsen et al., 1983).

Insulin secretion is influenced by diet composition, post prandial changes being greater in sheep fed a low compared with a high roughage diet (Trenkle, 1970; Weekes and Godden, 1981). Levels are also positively related to dietary crude protein supply to the small intestine (Bassett et al., 1971; Faichney and Weston, 1971; Weekes and Godden, 1981).

Physiological status may influence the insulin response to feeding as it has been reported that rate of insulin secretion is suppressed in lactating compared with non-lactating cows (Lomax et al., 1979). However Bines, Hart and Morant (1983) showed that the insulin response to feeding was greatest during early lactation and did not vary according to level of milk production.

A period of nutrient deprivation has been shown to result in a reduction in insulin levels (Trenkle, 1970; Bassett, 1974b).

Growth hormone

Feeding results in a decrease in GH concentration, for a period of up to 3 hours following feeding after which concentrations are restored to pre-feeding levels (Bassett et al., 1971; Bassett, 1974a and b; Bauman, Akers, Chapman, Tucker and Convey, 1979; Driver and Forbes, 1981). Negative relationships between GH concentration and nutrient intake and amount of organic matter and protein digested in the alimentary canal have been reported (Basset et al., 1971; Hove and Blom 1973; McDowell, 1983). In lactating cows, however, there was no marked decline in GH following feeding at any stage of lactation (Bines, Hart and Morant, 1983). In general, GH is not affected by the diet fed (Trenkle, 1970), although lower GH levels were reported in animals fed grain and hay compared with those fed only hay (Trenkle, 1971).

The effects of nutrient restriction on GH levels are variable. Although fasting does not necessarily increase GH concentration (Trenkle, 1970; Trenkle, 1978; Driver and Forbes, 1981), levels do tend to increase under conditions of nutrient insufficiency when animals are in certain physiological states, such as lactation (Cowie et al, 1980).

Prolactin

Increases in prolactin concentration in sheep have been reported following feeding; the magnitude of these increases is positively related to the level of feeding (Forbes et al., 1975; McAtee and Trenkle, 1971). No such effect was observed, however, in lactating goats (Hart, 1973) or cattle (Bauman, et al., 1979).

Prolactin levels are generally decreased during feed restriction (Trenkle, 1978; Sejrsen et al., 1983; Weekes and Godden, 1981).

Adrenal corticosteriods

Corticosteriod levels are not markedly or consistently influenced by feeding in sheep (Bassett, 1974b; Trenkle, 1978, Chesworth and Easdon,

1983). However, heifers fed a diet high in roughage tend to have lower corticosteroid levels compared with heifers fed a high concentrate diet (Mills and Jenny, 1979).

Nutrient restriction generally increases adrenal corticosteriod concentration (Mills and Jenny, 1979).

Thyroid hormones

There is no evidence of any marked effect of feeding on thyroid hormone concentration in sheep but in lactating suckler cows there is evidence that T^4 levels following feeding may be related to longer term energy balance (Coggins and Field, 1978).

Thyroid hormone concentration tends to decrease under conditions of nutrient restriction.

CIRCADIAN VARIATION

GH, Adrenal Cortisteroids and Prolactin

It has been shown that all these hormones fluctuate erratically during the day (Trenkle, 1978). A definate circadian rhythm has been demonstrated for adrenal corticosteroids (McNatty, Cashmore and Young, 1972; Chesworth and Easdon, 1983).

Thyroid hormones and Insulin

There is no evidence to suggest a circadian rhythm exists for these hormones.

IV. CONCLUSION

Milk production is under the control of many different factors including litter size, nutrition and genotype. These factors act through changes in circulating concentrations of many hormones. While the roles of individual hormones in relation to nutrient metabolism and milk yield have been studied, little is known of the way in which they interact to affect milk yield or of the effects of litter size and other factors on ewe hormone status. Work is required to characterise the effects of factors which control milk production on hormone profiles and their inter-relationships.

CHAPTER 3

EXPERIMENTAL MATERIALS AND METHODS

The experimental procedures carried out in each experiment were similar and so they are described in detail for the first experiment only; differences in procedure for subsequent experiments are indicated.

EXPERIMENT 1: MILK PRODUCTION AND ASSOCIATED ENDOCRINE AND BLOOD METABOLITE CONCENTRATIONS OF JANUARY LAMBING EWES REARING SINGLE OR TWIN LAMBS

ANIMALS AND MANAGEMENT

A group of 40 mature Border Leicester x Scottish Blackface (Greyface (GF)) ewes were selected during August 1982. On 17 August 1982, intravaginal progestagen sponges (Intervet Laboratories Ltd) were inserted. After 14 days the sponges were withdrawn, to synchronise oestrus, and all ewes were given an intra-muscular injection of 500 iu pregnant mares serum gonadotrophin (PMSG ; Intervet Laboratories Ltd), to induce ovulation. The dose of PMSG was selected in relation to the depth of anoestrus of the ewes at that time of year. Ewes were mated, at a ratio of 10 ewes per ram, with East Friesland (EF) rams which are known to be sexually active at this time of year. Rams were introduced to the ewes on 1 September 1982 at the first synchronised oestrus after pessary withdrawal, and remained with the ewes for 5 d.

Following mating all ewes were maintained as a group on pasture. Ewes were weighed and condition scored at regular intervals during pregnancy and fed supplementary concentrate feed and hay or silage, as required, to maintain the level of body condition in the group as uniform as possible. Six weeks prior to lambing, ewes were pregnancy diagnosed by X-ray. Barren ewes were removed from the group, leaving 29 ewes

in total which were housed in individual pens and received approximately 1 kg of dried grass pellets (160 g crude protein (CP)/kg DM) and 200 g of chopped hay/head/day. During the final two weeks of pregnancy the amount of dried grass pellets was gradually increased to 2 kg/head/day immediately prior to lambing.

Lambing was synchronised by induction of parturition (Bosc, 1972). After the first ewe had lambed, the remaining ewes were treated with 8 ml of a suspension containing 2 mg/ml betamethasone (Glaxovet Ltd). Treated ewes lambed over a period of 33 h. From the 29 ewes, 9 single lambs, 14 sets of twins, 5 sets of triplets and 1 set of quins were born. Ewes which were considered unsuitable for the subsequent lactation study owing to poor health were removed from the group. In order to obtain a group of 10 ewes rearing singles and a group of 10 ewes rearing twins it was necessary to remove one lamb from each of 3 twin and 3 triplet litters. In each case the smallest lamb of the litter was removed. Ewes and their lambs selected for the experiment were housed individually in 2 adjoining pens with a lamb creep gate which could be closed to separate ewes and lambs when required.

After lambing ewes were offered 3 kg of dried grass pellets and 200 g of hay/head/day, for the duration of the experimental period. The dried grass pellet had the following composition:-

Dry Matter g/kg	913
Organic Matter g/kg DM	917
Organic Matter digestibility +	0.67
Crude Protein (g/kg DM)	161
Gross Energy (MJ/kg DM)	17.5

+ based on in vitro analysis

Estimated ME intake and amino acid supply at the small intestine were calculated using derived constants detailed in ARC (1980; 1984).

Estimated ME intake = 19.6 MJ ME/day and amino acid supply to the small intestine = 48 g/day, assuming a digestibility value = 0.51, a feeding level = 3.5 x maintenance value and degradability value = 0.4.

Lambs were offered approximately 200 g/head/day of a lamb creep feed (BOCM Silcock) containing 12.2 MJ ME/kg DM and 160 g CP/kg DM, from week 7 of lactation until the end of the 10 week lactation period.

EXPERIMENTAL PROCEDURES

Live weight and condition score

Ewe body condition score (Russel, Doney and Gunn, 1969) and ewe and lamb live weights were recorded at parturition and subsequently, at weekly intervals, up to week 10 of lactation.

Milk Production

Milk secretion rate of each ewe was estimated over a period of approximately 4 hours, using a method similar to that of McCance (1959) and discussed by Doney et al. (1979). At the beginning and the end of the measurement period an intravenous injection of 5 iu oxytocin (Intervet Laboratories Ltd) was followed by hand-milking. The quantity of milk produced during the 4 hour period when the lambs were separated from their dams was determined and used to calculate production over 24 h; a sub-sample was retained for analysis. Measurements began approximately 5 days after lambing and were repeated at weekly intervals for 10 weeks.

Blood sample collection

Blood samples were collected by jugular venepuncture, prior to feeding, on one day of each week throughout the experiment. On each occasion all animals were sampled at 20 min intervals for a period of 2 h. Plasma aliquots were pooled within each animal and week, and stored at -20°C prior to analysis. During weeks 2, 4 and 10 of lactation the

collection period was extended to 8h and samples were also assayed individually. Sample collection began at 08.00 and animals were fed at 11.00 hours in each case.

CHEMICAL ANALYSIS

Milk composition

Milk samples were analysed for total solids by dry matter determination, fat content by the Gerber method (British Standard 696, Part 2, 1969), ash content by incineration of the residue from total solids analyses, protein content by a Kjeldahl digestion procedure and lactose content by calculating the difference between total solids content and the sum of fat, protein and ash content for each sample. Gross energy measurements were carried out on a representative number of milk samples using a Gallenkamp adiabetic bomb calorimeter. Equations for the relationship between milk gross energy and fat content were derived by regression analysis.

Blood metabolite and hormone determinations

All weekly pooled plasma samples were analysed to determine glucose, non-esterified fatty acids (NEFA), 3-hydroxybutyrate (3-OHB), urea, albumin and total protein, insulin, growth hormone (GH), cortisol, prolactin, triiodothyronine (T3) and thyroxine (T4), concentrations. Plasma samples collected at 20 min intervals over the 8 h periods were analysed individually to determine insulin, GH, cortisol and prolactin concentrations.

Blood metabolite determinations

Weekly pooled plasma samples were analysed for glucose (Richardson, 1977), NEFA (Paterson, 1963), 3-OHB (Ziven and Snarr, 1973), urea (Wilcox, Carroll, Sterling, Davis and Ware, 1966), albumin (Spencer and Price, 1977) and total protein concentrations (Failing,

Buckley and Zak, 1960).

Hormone determinations

Insulin Plasma insulin concentrations were determined using a double antibody radioimmunoassay technique based on the method of Tindal, Knaggs, Hart and Blake (1978). Purified ox insulin standard (provided by Dr. G. Court, Wellcome Research Laboratories) was used with a guinea-pig antiserum to bovine insulin (Welcome Reageants Ltd), ^{125}I-labelled insulin (Amersham International plc) and a donkey antiserum to guinea-pig gamma globulin as a second antiserum (Guildhay Antisera). The diluent buffer was a 0.04 M sodium phosphate buffer, pH 7.2, containing 1.25 g/l egg albumin.

Cross reactivities of the antiserum with other pancreatic hormones are not available but are likely to be minimal in view of the highly purified nature of the immunogen (Dr. G. Court, personal communication). Sensitivity of the assay was 0.5 mU/l. Within and between assay coefficients of variation were 0.06 (n=20) and 0.08 (n=11) respectively.

Growth Hormone Plasma GH concentrations were determined using a double antibody radioimmunoassay technique based on the method of Hart, Flux, Andrews and McNeilly (1975), using purified ovine antigen (NIH-oGH-1-3), rabbit antiserum to ovine GH (NIH-oGH-2), ^{125}I-labelled GH (NIH-oGH-1-3) prepared by the 1,3,4,6-Tetrachloro-3α,6αdiphenyl Glycoluril (Iodogen) method of Salacinski, McLean, Sykes, Clement-Jones and Lowry (1981) and donkey antiserum to rabbit globulin (Scottish Antibody Production Unit). The diluent buffer was the same as that described for the insulin radioimmunoassay.

Cross reactivities of the antiserum with highly purified preparations of ovine prolactin, ovine luteinising hormone (NIH-LH-S1), ovine follicle stimulating hormone (NIH-FSH-S1) and ovine thyroid stimulating hormone

were < 0.01. Sensitivity of the assay was 0.4 ug/l. Within and between assay coefficients of variation were 0.09 (n=18) and 0.12 (n=6) respectively.

Prolactin Plasma prolactin concentrations were determined using a double antibody radioimmunoassay, based on the method of McNeilly and Andrews (1974), using purified ovine prolactin (NIH-oPRL-I-1), rabbit antiserum to ovine prolactin (NIH-oPRL-1), ^{125}I-labelled prolactin (NIH-oPRL-I-1) prepared using lactoperoxidase as the oxidising agent, and donkey antiserum to rabbit gamma globulin (Scottish Antibody Production Unit). The assay diluent was 0.075M sodium phosphate buffered saline, pH 7.5, containing 10 g/l bovine serum albumin. Two sets of standard solutions were prepared to accomodate the large range of prolactin values. Assay conditions were optimised for each set of standard preparations.

Cross reactivities of the antiserum with highly purified preparations of ovine GH, ovine lutenising hormone (NIH-LH-S1), ovine follicle stimulating hormone (NIH-FSH-S1) and ovine thyroid stimulating hormone were < 0.01. Sensitivity of the assay was 0.4 ug/l for the low range of standards and 25 mg/l for the high range of standards. Within and between assay coefficients of variation were 0.07 (n=17) and 0.11 (n=12) respectively.

Cortisol Plasma cortisol concentrations were determined using a diagnostic kit (Corning Scientific and Medical) based on the procedure of Al-Ansari, Perry, Smith and Landen (1982). The diluent buffer was 0.5 M sodium phosphate buffered saline, pH 7.4. Standard preparations were diluted 1:10 with buffer before use. Cross reactivities of the antiserum were as follows:

Cortisol, 1.0; prednisolone, 0.44; 11-Deoxycortisol, 0.05; corticosterone and dexamethasone < 0.01. Sensitivity of the assay was 0.63 ug/l.

Within and between assay coefficients of variation were 0.08 (n=18) and 0.11 (n=11) respectively.

Triiodothyronine and Thyroxine T3 and T4 concentrations were measured by diagnostic kit (Corning Scientific and Medical).

Cross reactivities of the antisera were:

	Triidothyronine	Thyroxine
	Cross Reactivity	
L-Triidothyronine	1.0	0.05
D-Triiodothyronine	0.72	0.01
Triiodothyroacetic acid	0.27	<0.01
Tetraiodothyroacetic acid	<0.01	0.03
D-Thyroxine	<0.01	1.0
L-Thyroxine	<0.01	1.0

Sensitivity of the T3 assay was 0.25 ug/l. Within and between assay coefficients of variation were 0.09 (n=10) and 0.09 (n=4) respectively. Sensitivity of the T4 assay was 12.5 ug/l. Within and between assay coefficients of variation were 0.07 (n=10) and 0.08 (n=6) respectively.

STATISTICAL ANALYSIS

Analysis of variance

Analysis of variance was carried out using Genstat release 4.04 Version 1 (Rothamsted Experimental Station, 1984).

Weekly pooled samples:

Overall mean weekly values, for each parameter measured, were analysed for differences with treatment using a nested design taking animals as units and week of lactation as sub-units. In cases where between animal analysis yielded a significant difference ($P < 0.05$), or almost so, a Students 't' test was carried out on a between animal basis at weeks 2, 4 and 10 of lactation. The choice of individual weeks was

based on the expected change in level of milk production with stage of lactation.

Samples collected frequently:

For each animal, means of samples 1 to 9 inclusive (pre-feeding period) and samples 13 to 25 inclusive (post-feeding period) were calculated. Differences in overall mean values with treatment were analysed both prior to and following feeding on all occasions. Effects of feeding and of stage of lactation were analysed for each treatment group. Adjacent selected weeks were compared to assess the effect of stage of lactation.

Samples 10, 11 and 12 were excluded from these statistical comparisons due to the fact that feeding resulted in markedly increased variability and changes in circulating levels of most of the parameters measured, during the transition period between the fed and unfed states.

Skew distribution of data

Where data yielded a skew distribution all values were transformed onto a log scale ($y^1 = \ln(y)$) prior to statistical analysis. This transformation was used to analyse prolactin data. When data sets contained values less than 1, each value in the data set was increased by 1 prior to transformation ($y^1 = \ln(y+1)$). This transformation was used for the analyses of 3-OHB, GH and cortisol.

For ease of interpretation mean values have been back transformed before presentation. However, standard errors of differences between means are expressed in transformed units for statistical comparison.

Regression analysis

Regression analysis was carried out to estimate energy values of milk samples from measurements of milk fat content using the Minitab statistical package, release 81.1 (Penn State University, 1981). Where a significantly better fit ($P < 0.05$) was obtained using different regression

equations for different treatment groups, equations are presented separately.

EXPERIMENT 2: MILK PRODUCTION AND ASSOCIATED ENDOCRINE AND BLOOD METABOLITE CONCENTRATIONS OF APRIL LAMBING EWES REARING SINGLE OR TWIN LAMBS.

ANIMALS AND MANAGEMENT

On 22 November 1982 intravaginal progestagen sponges were inserted into 36 mature GF ewes for a 14 d period to synchronise oestrus. Treatment of ewes with PMSG after sponge withdrawl was not necessary as spontaneous ovulation occurs at this time of year. Rams were introduced to the ewes on 7 December 1982. Approximately six weeks prior to lambing ewes were pregnancy diagnosed by ultrasonic scanning. Parturition was induced and all ewes lambed over a period of 48 hours. From the 26 ewes which were diagnosed pregnant, 9 sets of singles, 13 sets of twins, and 4 sets of triplets were born. In order to obtain groups of 10 suitable ewes rearing singles and 10 ewes rearing twins it was necessary to remove one lamb from each of 2 twin and 3 triplet litters and 2 lambs from one set of triplets. Following the start of the lactation study two ewes rearing twin lambs were found to be unsuitable owing to poor health and were subsequently removed from the experiment, leaving a total of 8 ewes rearing twin lambs.

EXPERIMENTAL PROCEDURES

All procedures were as described for experiment 1.

STATISTICAL ANALYSIS

All analyses were as described for experiment 1.

EXPERIMENT 3: MILK PRODUCTION AND ASSOCIATED ENDOCRINE AND BLOOD METABOLITE CONCENTRATIONS OF EWES IN RELATION TO CRUDE PROTEIN CONTENT OF THE DIET.

ANIMALS AND MANAGEMENT

During August 1983 54 mature GF ewes were mated with Suffolk rams as described for experiment 1. Intravaginal progestagen sponges

were inserted on 4 August 1983 and rams were introduced at pessary withdrawl on 18 August 1983. Ewes were treated with 500 iu PMSG at pessary withdrawl. At housing all ewes were fed on dried grass pellets containing 157 g CP/kg DM and hay. The feeding regime prior to parturition was the same as in experiment 1. Parturition was not induced, owing to lambing difficulties encountered in the previous two experiments. Ewes lambed over a period of 6 days. The 24 ewes diagnosed pregnant gave birth to 12 sets of singles 10 sets of twins, 1 set of triplets and 1 set of quadruplets. In order to obtain a group of 20 ewes all suckling twin lambs it was necessary to remove two lambs from the quadruplet litter and to foster a lamb on to each of 10 single litters.

The selected ewes were allocated randomly to one of two groups, and were fed 3 kg of a dried grass based pelleted diet designed to contain either 120 g CP/kg DM (Diet L) or 175 g CP/kg DM (Diet H). When analysed the diets formulated were found to contain 157 and 218 g CP/kg DM respectively, which is likely to be attributable to the fact that the dried grass contained a higher crude protein content than expected. The full chemical analysis of the diets was as follows:

	Diet L	Diet H
Dry Matter (g/kg)	913	914
Organic Matter (g/kg DM)	916	914
Organic Matter digestibility+	0.74	0.74
Gross Energy (MJ/kg DM)	17.0	17.0
Crude Protein (g/kg DM)	157	218

+ based on <u>in vitro</u> analysis.

The diet composition was as follows:-

Constituent (g/kg)	Diet L	Diet H
Dried Grass	700	700
Barley	200	100
Fish Meal	-	100
Molasses	50	50
Vitamins and Minerals	50	50

Estimated ME intake = 22 MJME/day and amino acid supply to the small intestine = 67 g/day (low) and 83 g/day (high), assuming a digestibility value = 0.59, a feeding level = 3.5 x maintenance value and a degradability value = 0.4.

Three ewes in total were removed from the experiment during the 10 week lactation, one ewe from each treatment group during week 1 of lactation, owing to rejection of its foster lamb and one ewe from the low protein diet group during week 7 of lactation owing to mastitis infection.

EXPERIMENTAL PROCEDURE

All procedures were as described in experiment 1.

STATISTICAL ANALYSIS

All analyses were as described for experiment 1.

EXPERIMENT 4: MILK PRODUCTION AND ASSOCIATED ENDOCRINE AND BLOOD METABOLITE PROFILES OF EWES IN RELATION TO EWE GENOTYPE.

ANIMALS AND MANAGEMENT

Groups of 24 mature EF and 25 mature Scottish Blackface (SBF) ewes were mated at a synchronised oestrus with rams of their own breed between 15-17 November 1983. Ewes were not treated with PMSG. SBF ewes were grazed over the winter as a single flock and EF ewes

were housed and group fed ad libitum hay and a fixed amount of concentrate. All ewes were housed in individual pens approximately 4 weeks prior to lambing and fed 1.2 kg of a pelleted diet consisting of a mixture of dried molassed sugar beet pulp, dried grass and minerals (West Cumberland Farmers) and 200 g of hay/head/day.

Lambing was completed over a period of 6 days without induction of parturition. From the 14 EF ewes diagnosed pregnant, 4 sets of singles, 6 sets of twins and 4 sets of triplets were born. In order to obtain a group of 10 ewes rearing twin lambs it was necessary to remove one lamb from each of 3 triplet litters and foster 1 EF lamb on to a ewe rearing a single lamb. From the 15 SBF ewes diagnosed pregnant, 2 ewes gave birth to singles and 13 ewes gave birth to twins. It was not necessary to adjust litter size in SBF ewes as 10 suitable ewes were obtained from the group producing twin lambs.

Following parturition ewes were offered dried molassed sugar beet/grass pellets ad libitum and 200 g of hay/head/day. Intake of the pelleted diet was recorded on a daily basis. The chemical analysis of the diet was as follows:-

Dry Matter (g/kg)	899
Organic Matter (g/kg DM)	879
Organic Matter digestibility+	0.67
Gross Energy (MJ/kg DM)	14.6
Crude Protein (g/kg DM)	164

+ based on in vitro analysis.

The experiment was carried out during the first 14 weeks of lactation. One ewe of each breed was removed from the experiment during the 14 week lactation, owing to health problems. The EF ewe was removed during week 11 of lactation and the SBF ewe during week 14.

EXPERIMENTAL PROCEDURES

All procedures were as described for experiment 1 and were carried out for the duration of the 14 week lactation study. The extended periods of blood sampling (i.e. over 8 h) were carried out during weeks 2,4,10 and 14 of lactation.

STATISTICAL ANALYSIS

All analyses were as described for experiment 1. Weeks 2, 6 and 14 were selected for the additional comparisons.

CHAPTER 4

THE EFFECT OF LAMB NUMBER (OR SUCKLING STIMULUS) AND SEASON OF LAMBING (EXPTS. 1 and 2)

RESULTS

MILK PRODUCTION

In both the January- and April-lambing experiments balanced groups of ewes rearing single or twin lambs were made, in some cases, up by removal of a lamb. It was not possible to randomise treatment groups in terms of foetal number. Therefore the effect of number of lambs born was examined in relation to daily milk production during the first week of lactation using combined data from both experiments. There was no significant difference in daily milk yield in ewes which bore twin lambs and suckled singles (mean=2.70 kg/day; n=3; s.e.=0.252) compared with ewes which bore and suckled single lambs (mean=2.82; n=13; s.e.=0.300). Similiarly there was no difference between ewes which bore triplet lambs and suckled twins (mean=3.74 kg/day; n=6; s.e.=0.594) and ewes which bore and suckled twin lambs (mean=3.40 kg/day ; n=12; s.e. = 0.261).

Mean daily milk yield was maximum during week 1 of the 10 week lactation and generally declined throughout lactation in ewes suckling single lambs (S ewes) and in ewes suckling twin lambs (T ewes), at each of the lambing seasons (Figure 1). The rate at which milk yield declined was generally lower in S ewes compared with T ewes.

Overall mean daily milk yield (i.e. grand mean for the whole 10 week lactation) was lower in S ewes than in T ewes at each lambing season, the difference being significant in the January-lambing group (1.93 v. 2.33 kg/day; s.e.d.= 0.110; $P < 0.01$) but not in the April-lambing group (1.95 v. 2.28 kg/day; s.e.d.=0.168; $P > 0.05$). The means were tested at 3 individual weeks (i.e. weeks 2, 4 and 10 of lactation). The

Figure 1. Mean daily milk production (kg/day) during lactation in S and T ewes at two seasons of year (January: single x——x, twin o——o; s.e.d. weeks 2, 4 and 10 = 0.183, 0.191, 0.181. April: single x- - -x, twin o- - -o; s.e.d. weeks 2, 4 and 10 = 0.280, 0.161, 0.150).

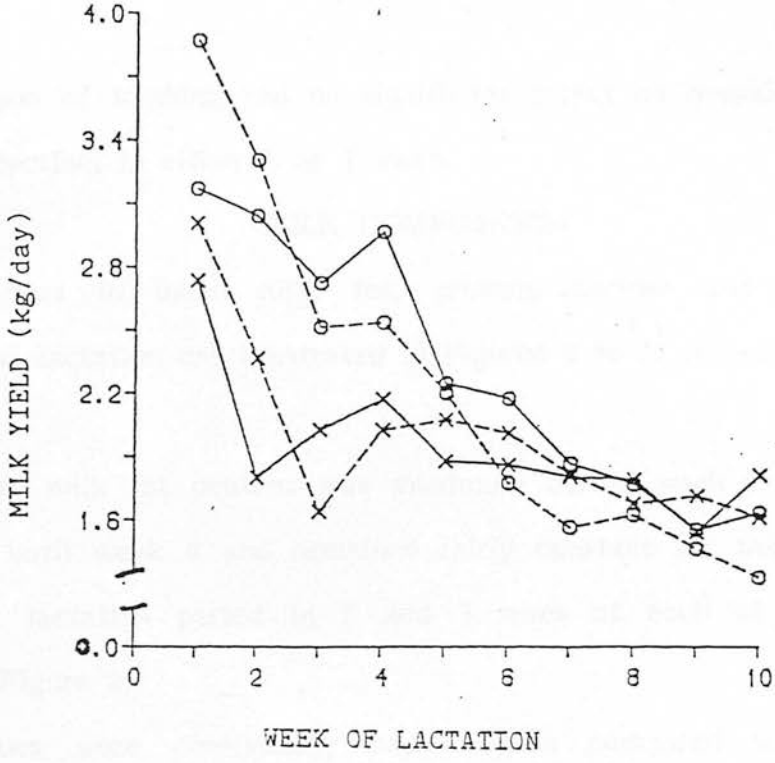
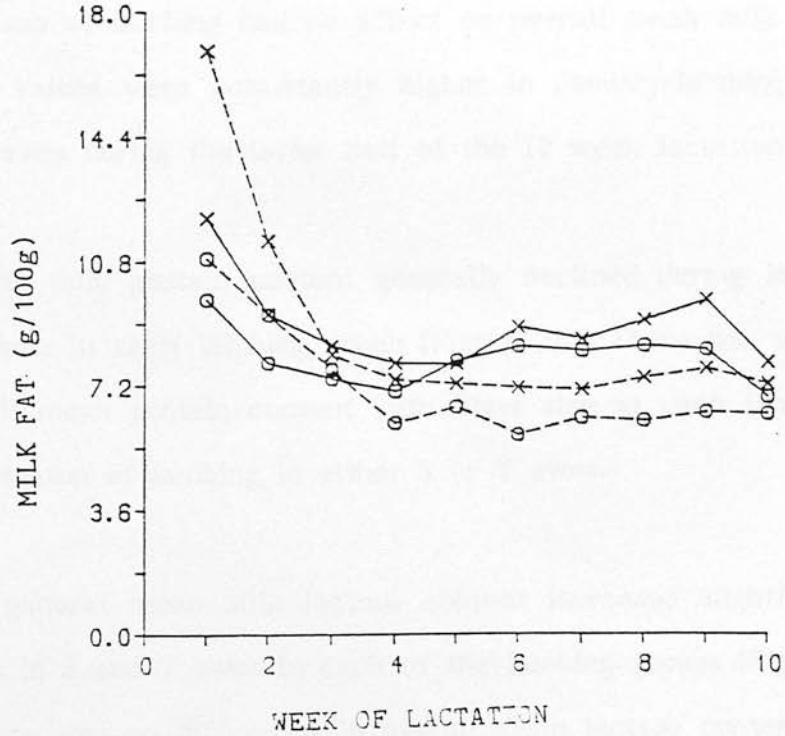


Figure 2. Mean milk fat contents (g/100 g) during lactation in S and T ewes at two seasons of year (January: single x——x, twin o——o; overall s.e.d. = 0.711. April: single x- - -x, twin o- - -o; s.e.d. weeks 2, 4 and 10 = 1.049, 0.633, 0.484).



mean yield in S ewes was significantly lower than in T ewes during weeks 2 and 4 ($P < 0.001$) but not week 10 of lactation, in each lambing group.

Season of lambing had no significant effect on overall mean daily milk production, in either S or T ewes.

MILK COMPOSITION

Changes in mean milk fat, protein, lactose and ash content throughout lactation are illustrated in Figures 2 to 5, respectively.

Fat

Mean milk fat content was maximum during week 1 of lactation, declined until week 4 and remained fairly constant for the rest of the 10 week lactation period in S and T ewes at each of the lambing seasons (Figure 2).

Values were consistently higher in S compared with T ewes, although overall mean values differed significantly only in the April-lambing group (8.75 v. 7.14 g/100 g; s.e.d.=0.581; $P < 0.05$). In the April group values did not differ significantly at any of the 3 tested weeks.

Season of lambing had no effect on overall mean milk fat content, although values were consistently higher in January-lambing than April-lambing ewes during the latter half of the 10 week lactation study.

Protein

Mean milk protein content generally declined during lactation in S and T ewes in each lambing group (Figure 3). There was no difference in overall mean protein content with litter size at each lambing season, or with season of lambing in either S or T ewes.

Lactose

In general mean milk lactose content increased slightly throughout lactation in S and T ewes in each of the lambing groups (Figure 4).

There was no difference in overall mean lactose content with litter size in either of the lambing groups.

Figure 3. Mean milk protein contents (g/100 g) during lactation in S and T ewes at two seasons of year (January: single x——x, twin o——o; overall s.e.d. = 0.119. April: single x- - -x, twin o- - -o; overall s.e.d. = 0.115).

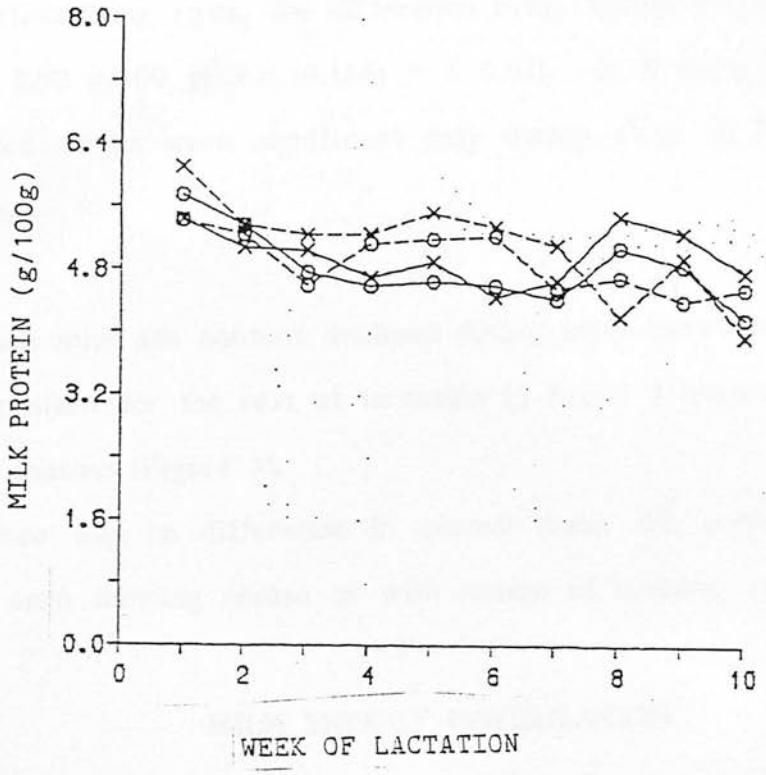
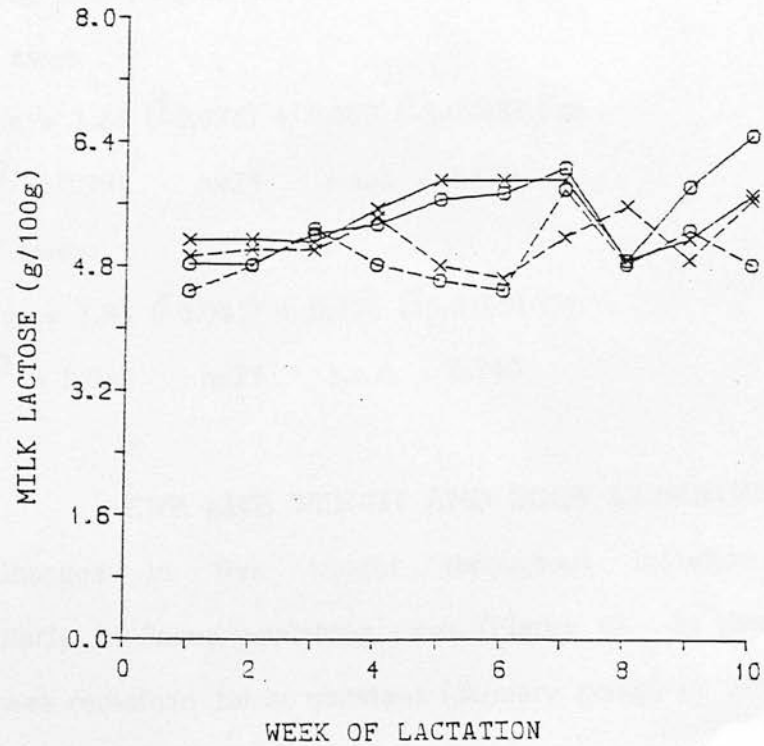


Figure 4. Mean milk lactose contents (g/100 g) during lactation in S and T ewes at two seasons of year (January: single x——x, twin o——o; overall s.e.d. = 0.121. April: single x- - -x, twin o- - -o; overall s.e.d. = 0.220).



Season of lambing had a significant effect on overall mean content. January-lambing ewes produced milk of higher lactose content compared with April-lambing ewes, the difference being significant in T ewes only (5.49 v. 4.92 g/100 g;s.e.d.=0.166; $P < 0.01$). In T ewes, differences at the tested weeks were significant only during week 10 ($P < 0.001$) of lactation.

Ash

Mean milk ash content declined during early lactation and remained fairly constant for the rest of lactation in S and T ewes at each of the lambing seasons (Figure 5).

There was no difference in overall mean ash content with litter size in each lambing season or with season of lambing in either S or T ewes.

MILK ENERGY CALCULATION

Throughout lactation the measured milk energy (Em) production (MJ/kg DM) in S and T ewes (data combined from both lambing groups) was closely and linearly related to milk fat (Fm) content (g/100 g). The linear regression equations were as follows:

1. S ewes

   $Em = 1.69 (\pm 0.075) + 0.407 (\pm 0.0068) Fm$

   $r^2 = 0.994$ n=25 r.s.d. = 0.170

2. T ewes

   $Em = 1.96 (\pm 0.085) + 0.371 (\pm 0.0100) Fm$

   $r^2 = 0.984$ n=25 r.s.d. = 0.140

EWE LIVE WEIGHT AND BODY CONDITION

Changes in live weight throughout lactation were erratic particularly in January-lambing ewes (Figure 6). In general live weight in S ewes remained fairly constant (January group) or increased

Figure 5. Mean milk ash contents (g/100 g) during lactation in S and T ewes at two seasons of year (January: single x——x, twin o——o; overall s.e.d. = 0.026. April: single x– – –x, twin o– – –o; overall s.e.d. = 0.026)

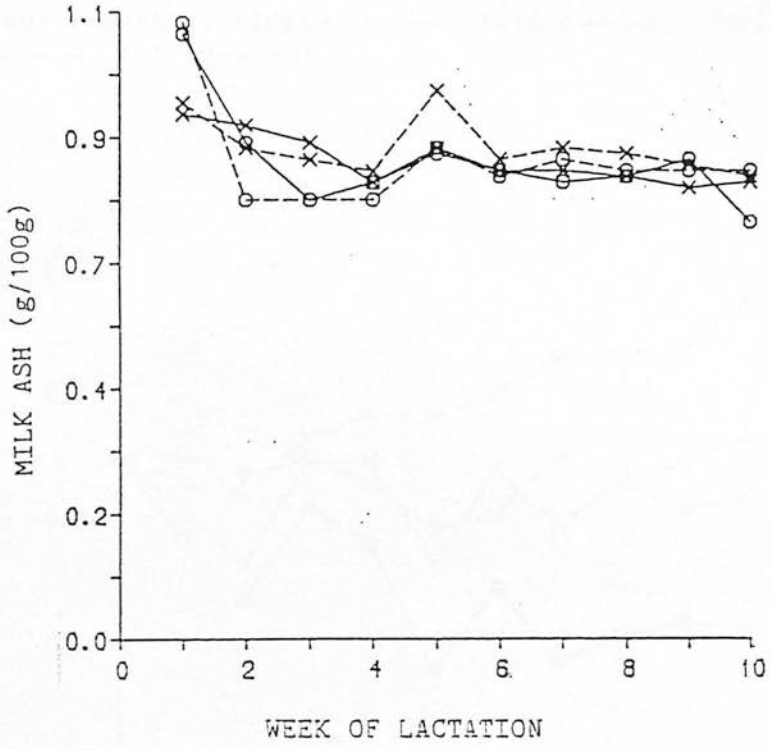
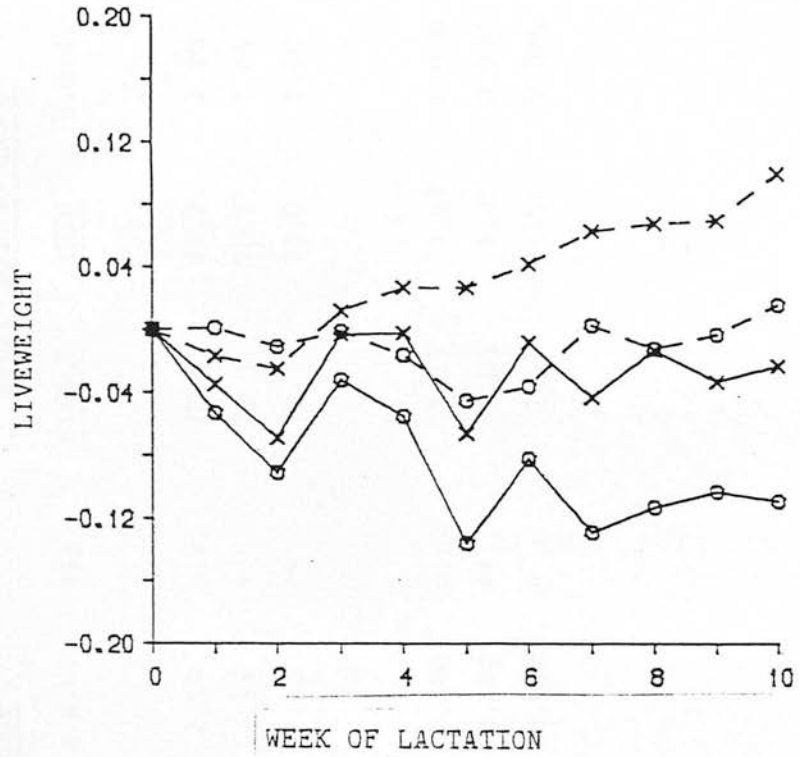


Figure 6. Proportional changes in ewe live weight and body condition score during lactation in S and T ewes at two seasons of year (January: single x——x, twin o——o. April: single x– – –x, twin o– – –o)



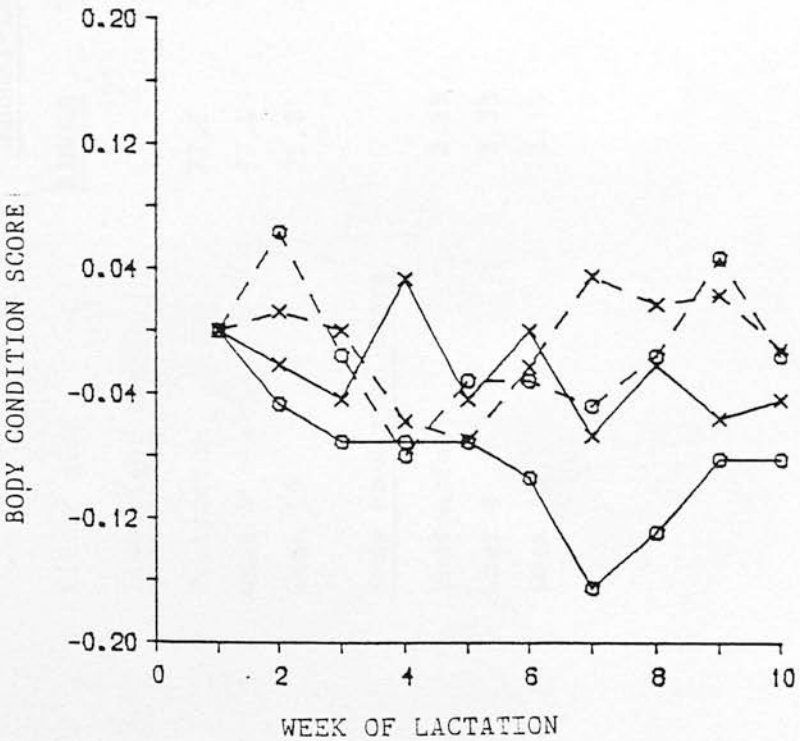


Table 1. Mean ewe live weights (kg) and body condition scores, postpartum and during weeks 4 and 10 of lactation.

	JANUARY-LAMBING GROUP				APRIL-LAMBING GROUP			
Litter size	SINGLE	TWIN	s.e.d.	sig.	SINGLE	TWIN	s.e.d.	sig.
Liveweight (kg)								
Postpartum	77.6	75.0	2.55	n.s.	70.6	71.9	2.80	n.s.
Week 4	77.4	70.8	2.51	*	72.5	70.7	2.04	n.s.
Week 10	75.8	66.8	2.60	**	77.7	73.0	2.10	*
Body condition score								
Postpartum	2.25	2.13	0.100	n.s.	2.15	1.97	0.090	n.s.
Week 4	2.33	1.98	0.102	**	2.03	1.81	0.062	**
Week 10	2.15	1.95	0.083	*	2.13	1.94	0.084	*

throughout lactation (April group), while T ewes tended to lose weight during early lactation and either remained fairly constant (January group) or gained live weight (April group) during late lactation. Patterns of body condition score change throughout lactation were broadly similar to changes in live weight (Figure 6).

There was no effect of litter size on mean post partum live weight or body condition score in either lambing group (Table 1). Mean live weight was significantly higher in S compared with T ewes during weeks 4 ($P < 0.05$) and 10 ($P < 0.01$) of lactation in the January-lambing group and during week 10 ($P < 0.05$) of lactation in the April-lambing group. Body condition score was significantly higher in S than T ewes during weeks 4 ($P < 0.01$) and 10 ($P < 0.05$) of lactation at each lambing season.

Post partum liveweight was higher in January ewes than in April ewes although the difference was significant only in S ewes ($P < 0.05$). Body condition score was generally higher in January-lambing ewes compared with April-lambing ewes, differences being significant at week 4 of lactation in S ($P < 0.01$) and T ewes ($P < 0.05$).

LAMB LIVE WEIGHT

Mean birth weight was slightly lower in lambs suckled as twins compared with lambs suckled as singles (Table 2), this difference being significant ($P < 0.01$) in the January-lambing group. Rate of lamb live weight gain was significantly greater in lambs suckled as singles compared with lambs suckled as twins during lactation and this was reflected in a highly significant difference in live weight during week 10 of lactation ($P < 0.001$) in each of the lambing groups.

Season of lambing had a significant effect on the rate of live weight gain. Values were higher in the January-lambing group compared

Table 2. Mean lamb live weights (kg), at birth and 10 weeks of age and mean live weight gains (g/day) during 0-4 and 4-10 weeks of lactation.

	JANUARY-LAMBING GROUP				APRIL-LAMBING GROUP			
Litter size	SINGLE	TWIN	s.e.d.	sig.	SINGLE	TWIN	s.e.d.	sig.
Live weight (kg)								
Birth	5.5	4.8	0.22	**	5.5	5.0	0.33	n.s.
10 weeks	27.3	21.7	0.73	***	28.9	23.5	1.03	***
Live weight gain (g/day)								
0-4 weeks	377	297	16.2	***	322	272	19.6	*
4-10 weeks	329	254	14.4	***	396	305	15.4	***

with the April-lambing group, in lambs suckled as singles, during weeks 0-4 of lactation ($P < 0.05$). In contrast values were higher in the April-lambing group compared with the January group in both single- and twin-suckled lambs during weeks 4-10 of lactation ($P < 0.001$).

WEEKLY POOLED BLOOD METABOLITE CONCENTRATION

Glucose

Mean plasma glucose concentration tended to increase slightly throughout lactation in S and T ewes in each lambing group (Figure 7). There was no difference in overall mean glucose concentration with litter size in either of the lambing groups or with season of lambing in either S or T ewes.

Non-esterified fatty acids (NEFA)

Circulating NEFA concentration in S ewes increased to a peak between week 6 and 7 of lactation and declined thereafter while in T ewes values were elevated during early lactation up to week 3 or 4 of lactation, before declining during the remainder of lactation (Figure 8).

There was no significant effect of litter size on overall mean NEFA concentration in either the January- or April-lambing group. However, when individual weeks were tested NEFA levels were lower in S ewes compared with T ewes during weeks 2 ($P < 0.001$) and 4 ($P < 0.01$) of lactation in January-lambing ewes and during week 2 ($P < 0.01$) of lactation in April ewes. It is noteworthy that during mid-lactation levels were in fact higher in S ewes than in T ewes.

Season of lambing had no effect on overall mean NEFA concentration when ewes were rearing twin lambs although the value was significantly lower in single rearing January-lambing ewes compared with single rearing April-lambing ewes (1215 *v.* 1506 uM/l; s.e.d.=106.2; $P < 0.05$). In S ewes values in January-lambing ewes were significantly lower than in April ewes during weeks 2 ($P < 0.01$) and 4 ($P < 0.001$) but not during week 10 of lactation.

Figure 7. Mean plasma glucose concentrations (mM/1) during lactation in S and T ewes at two seasons of year (January: single x——x, twin o——o; overall s.e.d. = 0.055. April: single x- - -x, twin o- - -o; overall s.e.d. = 0.144)

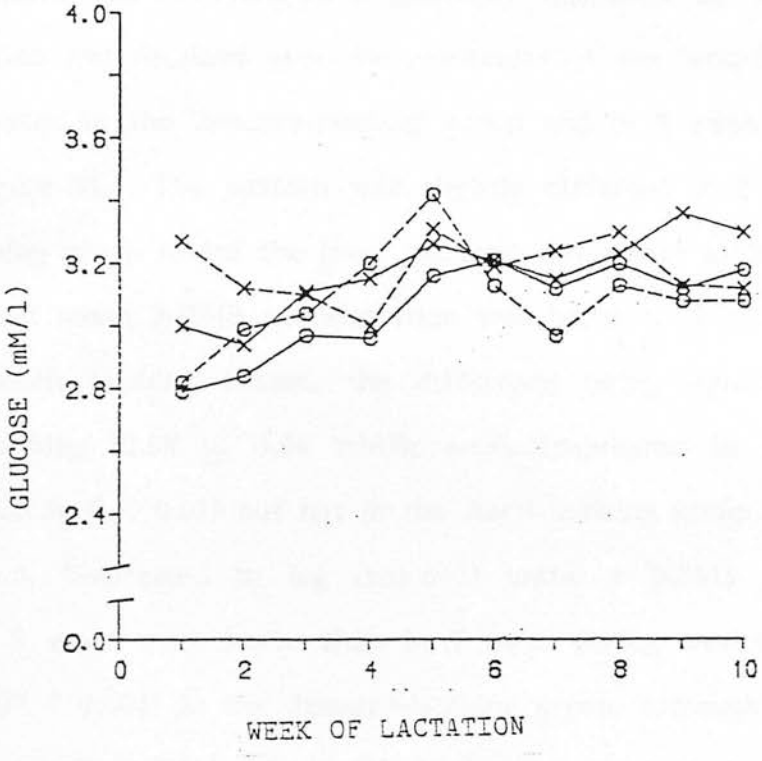
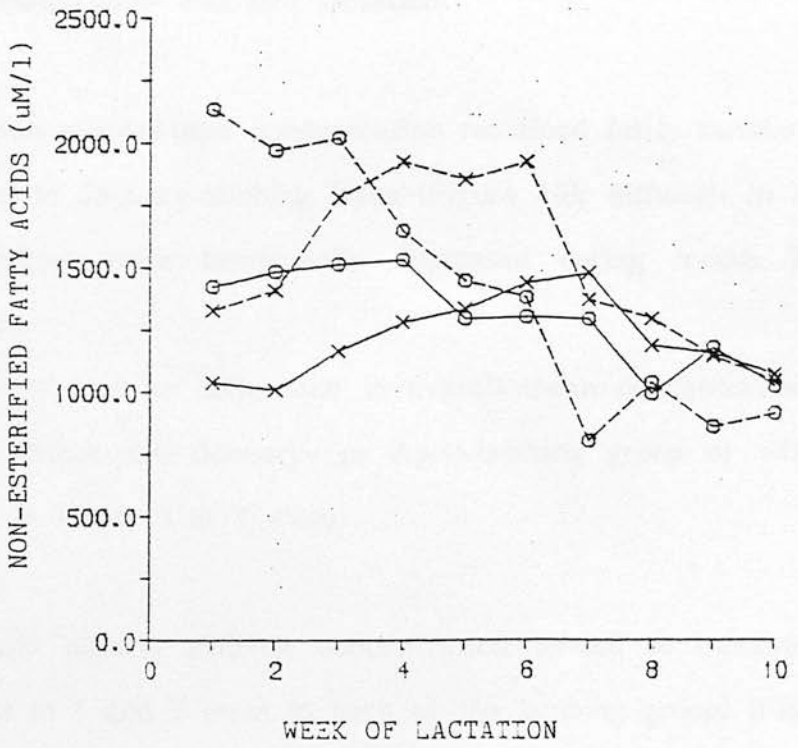


Figure 8. Mean plasma NEFA concentrations (uM/1) during lactation in S and T ewes at two seasons of year (January: single x——x, twin o——o; s.e.d. weeks 2,4 and 10 = 83.4, 83.1, 135.3. April: single x- - -x, twin o- - -o; s.e.d. weeks 2, 4 and 10 = 170.0, 204.3, 191.5)



3-hydroxybutyrate (3-OHB)

Plasma 3-OHB concentration generally increased up to a peak in mid-lactation and declined over the remainder of the lactation period in S and T ewes in the January-lambing group and in S ewes in the April group (Figure 9). The pattern was slightly different in T ewes in the April-lambing group where the level declined throughout lactation.

Overall mean 3-OHB concentration was lower in S ewes than in T ewes at each lambing season, the difference being significant in the January-lambing (0.68 v. 0.94 mM/l; s.e.d. (expressed in log (value+1) units) = 0.045; $P < 0.01$) but not in the April-lambing group (0.60 v. 0.69 mM/l; s.e.d. (expressed in log (value+1) units) = 0.061; $P > 0.05$). Values in S ewes were lower than in T ewes during weeks 2 and 4 of lactation ($P < 0.001$) in the January-lambing group, although during week 2 of lactation ($P < 0.01$) only in the April group.

Season of lambing had no effect on overall mean 3-OHB concentration, although levels were consistently higher in the January-lambing compared with the April-lambing group in each of the rearing groups during mid- and late lactation.

Urea

Mean plasma urea concentration remained fairly constant throughout lactation in January-lambing ewes (Figure 10), although in April-lambing ewes values were temporarily depressed during weeks 2 and 3 of lactation.

There was no difference in overall mean concentration with litter size in either the January- or April-lambing group or with season of lambing in either S or T ewes.

Albumin

Mean plasma albumin concentration tended to increase throughout lactation in S and T ewes in each of the lambing groups (Figure 11).

Figure 9. Back-transformed mean plasma 3-OHB concentrations (mM/1) during lactation in S and T ewes at two seasons of year (January: single x——x, twin o——o; s.e.d. (expressed in log (value + 1) units) weeks 2, 4 and 10 = 0.056, 0.062, 0.046. April: single x– – –x, twin o– – –o; s.e.d. (expressed in log (value + 1) units) weeks 2, 4 and 10 = 0.098, 0.085, 0.032)

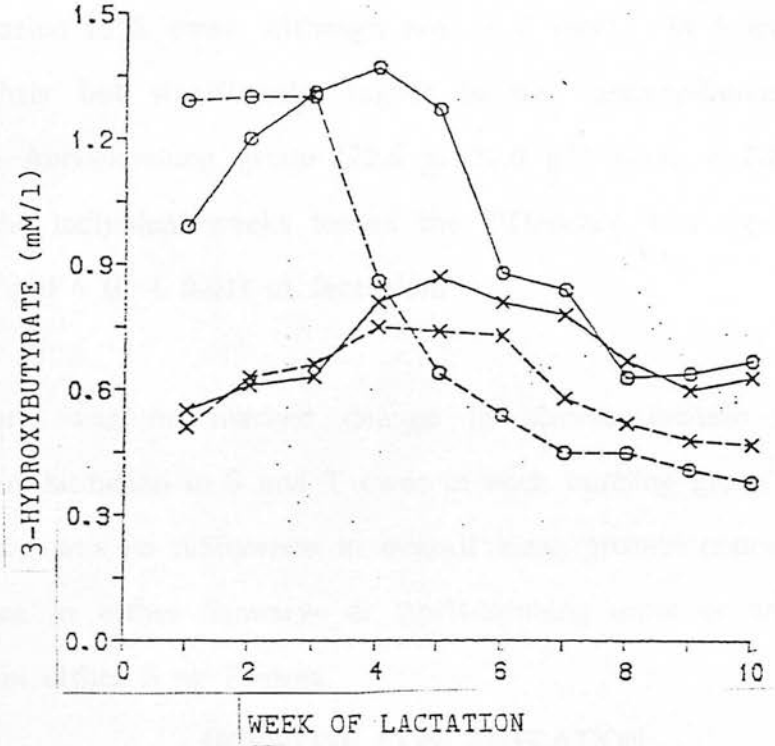
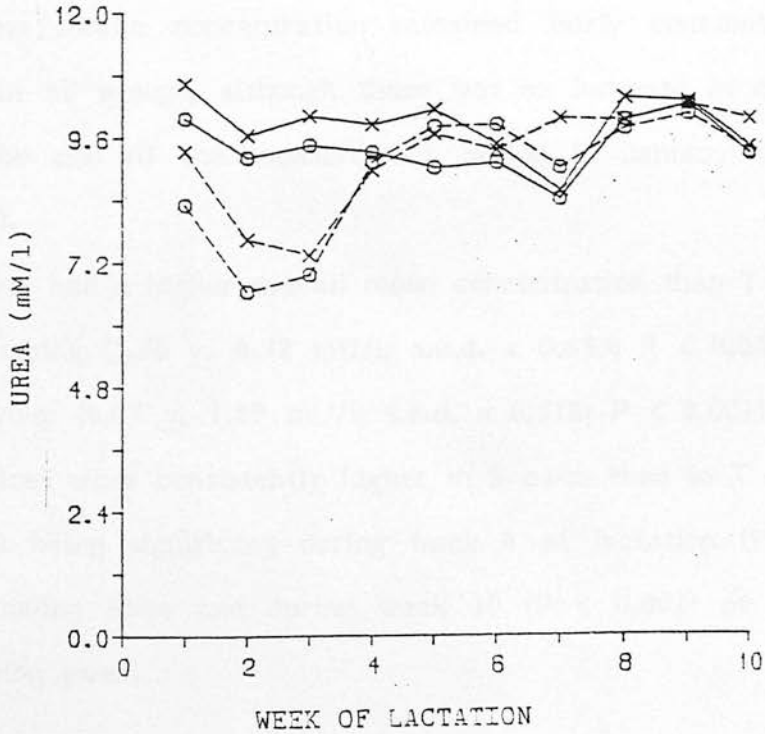


Figure 10. Mean plasma urea concentrations (mM/1) during lactation in S and T ewes at two seasons of year (January: single x——x, twin o——o; overall s.e.d. = 0.369. April: single x– – –x, twin o– – –o; overall s.e.d. = 0.338)



There was no difference in overall mean concentration with litter size in either the January- or April-lambing group.

Season of lambing had a significant effect on the overall mean concentration in S ewes, although not in T ewes. In S ewes the value was slightly but significantly higher in the January-lambing compared with the April-lambing group (22.6 v. 22.0 g/l; s.e.d. = 0.27; $P < 0.05$) and in the individual weeks tested the difference was significant during weeks 2 and 4 ($P < 0.01$) of lactation.

Protein

There was no marked change in plasma protein concentration throughout lactation in S and T ewes in each lambing group (Figure 12).

There was no difference in overall mean protein concentration with litter size in either January- or April-lambing ewes or with season of lambing in either S or T ewes.

HORMONE CONCENTRATION

Insulin

Weekly pooled samples:

Plasma insulin concentration remained fairly constant throughout lactation in all groups, although there was an increase in concentration towards the end of the measurement period in January-lambing ewes (Figure 13).

S ewes had a higher overall mean concentration than T ewes in the January-lambing (5.70 v. 4.32 mU/l; s.e.d. = 0.633; $P < 0.05$) and April-lambing group (4.07 v. 1.89 mU/l; s.e.d. = 0.518; $P < 0.001$). Individual weekly values were consistently higher in S ewes than in T ewes, tested differences being significant during week 4 of lactation ($P < 0.05$) in January-lambing ewes and during week 10 ($P < 0.001$) of lactation in April-lambing ewes.

Figure 11. Mean plasma albumin concentrations (g/l) during lactation in S and T ewes at two seasons of year (January: single x——x, twin o——o; overall s.e.d. = 0.39. April: single x- - -x, twin o- - -o; overall s.e.d. = 0.33)

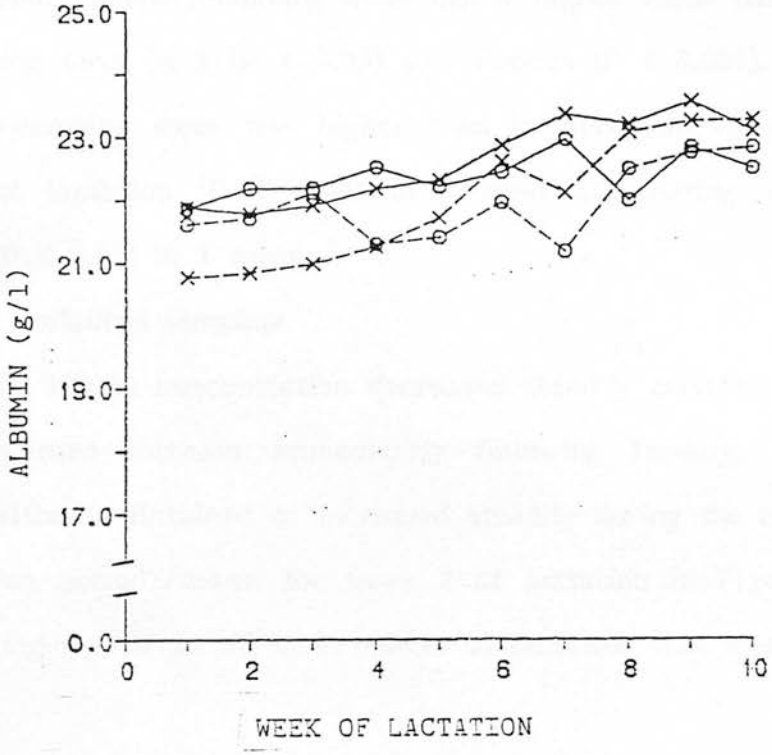
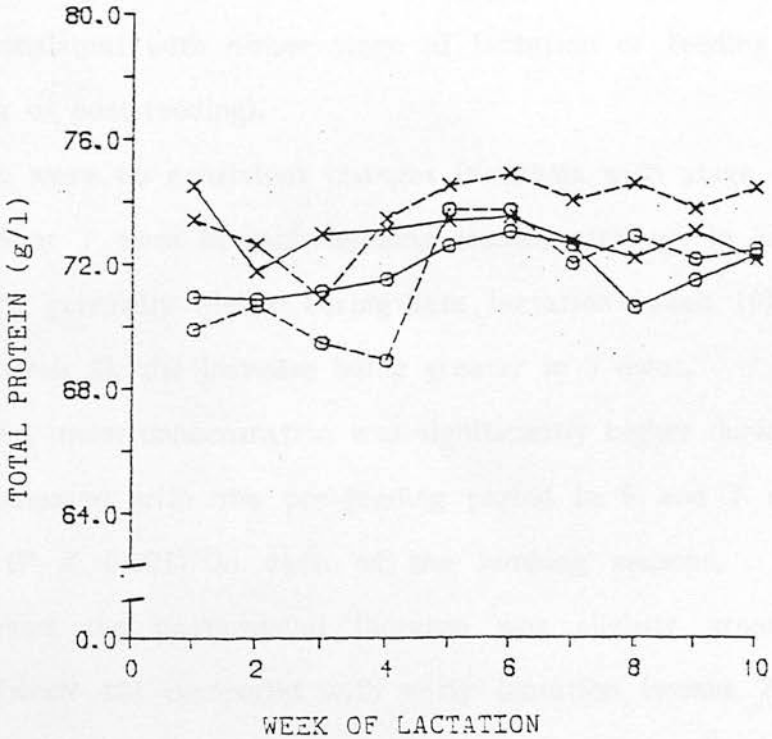


Figure 12. Mean plasma protein concentrations (g/l) during lactation in S and T ewes at two seasons of year (January: single x——x, twin o——o; overall s.e.d. = 0.64. April: single x- - -x, twin o- - -o; overall s.e.d. = 1.31)



Season of lambing had a significant effect on overall mean concentration. January-lambing ewes had a higher value compared with April-lambing ewes in S ($P < 0.05$) and T ewes ($P < 0.001$). The value in January-lambing ewes was higher than in April-lambing ewes during week 4 of lactation ($P < 0.05$) in S ewes and during week 10 of lactation ($P < 0.05$) in T ewes.

Frequently collected samples:

Plasma insulin concentration decreased steadily prior to feeding and after a marked increase immediately following feeding, levels were generally either maintained or increased steadily during the remainder of the sampling period (shown for week 2 of lactation in Figure 14), the pattern being similar at all three stages of lactation (i.e. weeks 2, 4 and 10).

Overall mean concentration based on 20 minute sampling intervals before (samples 1-9) and after (samples 13-25) feeding was consistently higher in S compared with T ewes on all occasions in each lambing season (Table 3), although the level of significance of these differences was not consistent with either stage of lactation or feeding status (i.e. pre-feeding or post-feeding).

There were no consistent changes in values with stage of lactation in either S or T ewes in each lambing season, although in January ewes values were generally higher during late lactation (week 10) than early lactation (week 2), the increase being greater in S ewes.

Overall mean concentration was significantly higher during the post-feeding compared with the pre-feeding period in S and T ewes on all occasions ($P < 0.001$) in each of the lambing seasons. In January-lambing ewes the postprandial increase was slightly greater in late lactation (week 10) compared with early lactation (weeks 2 and 4), in both S and T ewes, although a similar trend was not observed in April ewes.

Figure 13. Mean plasma insulin concentrations (mU/l) during lactation in S and T ewes at two seasons of year (January: single x——x, twin o——o; s.e.d. weeks 2, 4 and 10 = 1.043 1.116,0.932. April: single x- - -x, twin o- - -o; s.e.d. weeks 2, 4 and 10 = 0.870, 0.892, 0.668).

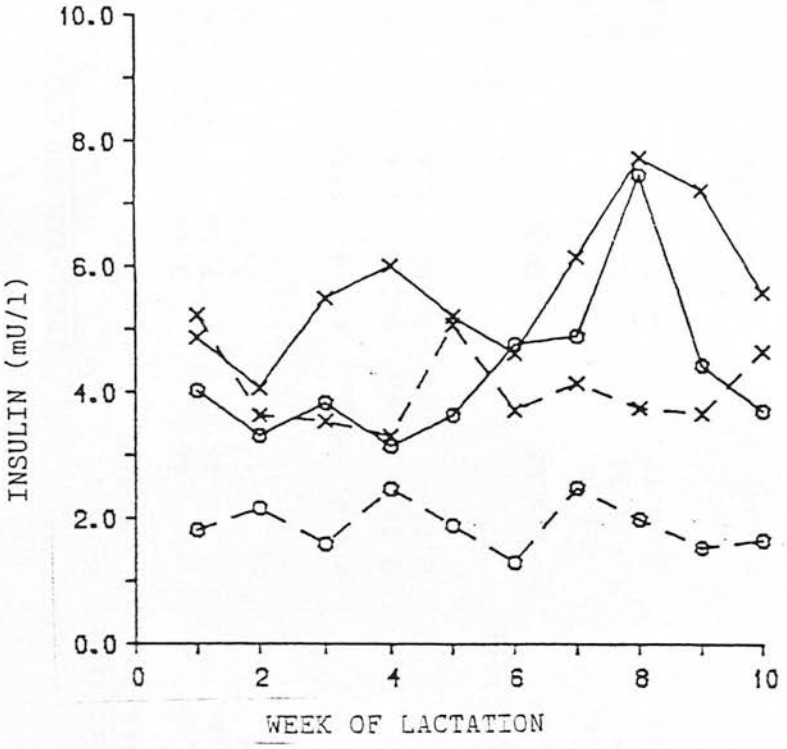


Figure 14. Changes in mean plasma insulin concentration (mU/l) during an 8 hour sampling period at week 2 of lactation in S and T ewes at two seasons of year (January: single x——x, twin o——o. April: single x- - -x, twin o- - -o)

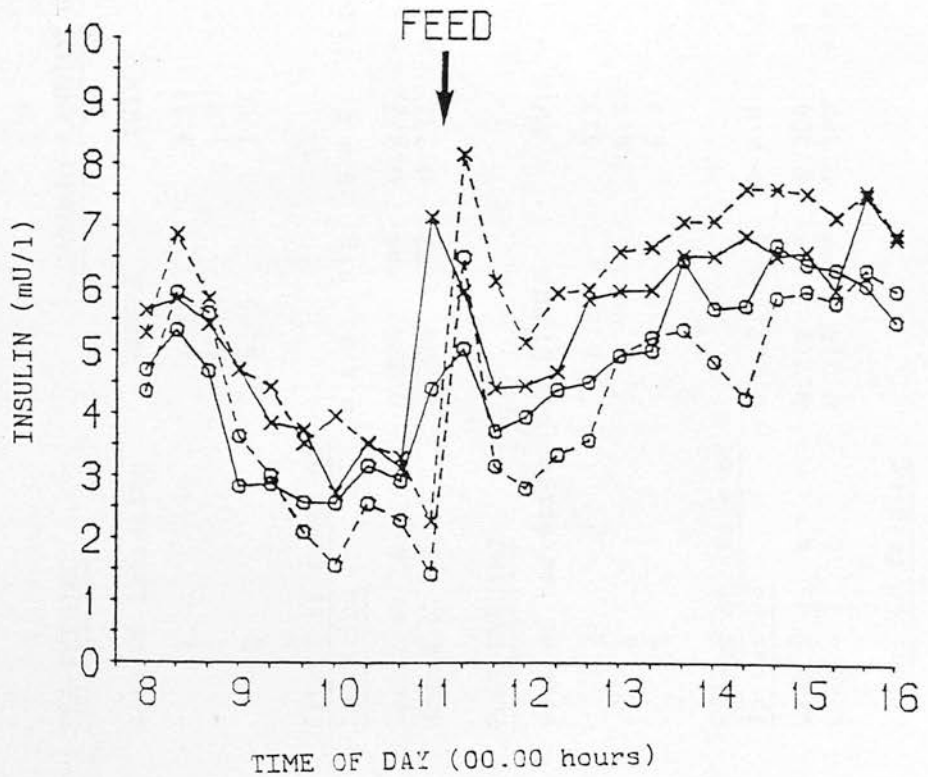


Table 3. Overall mean insulin concentrations (mμ/l) during the pre-feeding (samples 1-9) and post-feeding (samples 13-25) periods in S and T ewes during weeks 2, 4 and 10 of lactation at two seasons of year (January and April).

PRE-FEEDING	JANUARY-LAMBING GROUP				APRIL-LAMBING GROUP			
			Effect of litter size				Effect of litter size	
Week of lactation	SINGLE	TWIN	s.e.d.	sig.	SINGLE	TWIN	s.e.d.	sig.
2	4.28	3.51	0.969	n.s.	4.60	3.44	0.701	n.s.
4	5.14	3.87	0.813	n.s.	4.45	3.14	0.580	*
10	6.05	3.94	0.823	*	4.77	3.24	0.789	n.s.

Effect of stage of lactation	s.e.d.	sig.	s.e.d.	sig.	s.e.d.	sig.	s.e.d.	sig.
Week 2 v. 4	0.224	***	0.277	n.s.	0.243	n.s.	0.248	n.s.
Week 4 v. 10	0.244	***	0.231	n.s.	0.246	n.s.	0.362	n.s.

POST-FEEDING			Effect of litter size				Effect of litter size	
Week of lactation	SINGLE	TWIN	s.e.d.	sig.	SINGLE	TWIN	s.e.d.	sig.
2	6.20	5.52	0.909	n.s.	6.86	4.95	0.658	*
4	6.99	4.98	0.881	*	7.20	5.49	0.720	*
10	8.78	6.71	1.031	n.s.	6.77	4.55	0.600	**

Effect of stage of lactation	s.e.d.	sig.	s.e.d.	sig.	s.e.d.	sig.	s.e.d.	sig.
Week 2 v. 4	0.212	***	0.204	*	0.221	n.s.	0.239	*
Week 4 v. 10	0.214	***	0.289	***	0.265	n.s.	0.237	***
Effect of feeding								
Week 2	0.235	***	0.240	***	0.226	***	0,294	***
Week 4	0.222	***	0.244	***	0.323	***	0.336	***
Week 10	0.243	**	0.251	***	0.262	***	0.282	***

Growth hormone (GH)

Weekly pooled samples:

Plasma GH concentration remained fairly constant during lactation in S and T ewes in each lambing season (Figure 15); between week variation was large, particularly in T ewes.

There was a significant difference with litter size in overall mean GH concentration. S ewes had a lower value than T ewes in the January-lambing group (2.70 v. 5.88 ug/l; s.e.d. (expressed in log (value+1) units = 0.173; $P < 0.01$) and the April-lambing group (2.90 v. 6.70 ug/l; s.e.d. (expressed in log (value+1) units = 0.254; $P < 0.05$). Individual weekly values were consistently lower in S ewes compared with T ewes and in the tested weeks the difference was significant, in January-lambing ewes during weeks 2 ($P < 0.05$), 4 ($P < 0.01$) and 10 ($P < 0.001$) of lactation and in April-lambing ewes during weeks 4 ($P < 0.05$) and 10 ($P < 0.01$) of lactation.

Season of lambing had no effect on overall mean GH concentration in either S or T ewes.

Frequently collected samples:

Mean GH concentration fluctuated throughout the sampling period in all groups showing no consistent trend at each of the three periods (shown for week 2 of lactation in Figure 16).

There was a highly significant difference with litter size in overall mean concentration before and after feeding on all occasions in each lambing season (Table 4). Levels were lower in S ewes compared with T ewes.

Changes in overall mean values with stage of lactation were not consistent although values in late lactation (week 10) tended to be lower than in early lactation (week 2) particularly during the post-feeding period.

Figure 15. Back-transformed mean plasma GH concentrations (ug/l) during lactation in S and T ewes at two seasons of year (January: single x——x, twin o——o; s.e.d. (expressed in log (value + 1) units) weeks 2, 4 and 10 = 0.222, 0.210, 0.244. April: single x- - -x, twin o- - -o; s.e.d. (expressed in log (value + 1) units) weeks 2, 4 and 10 = 0.277, 0.324, 0.177)

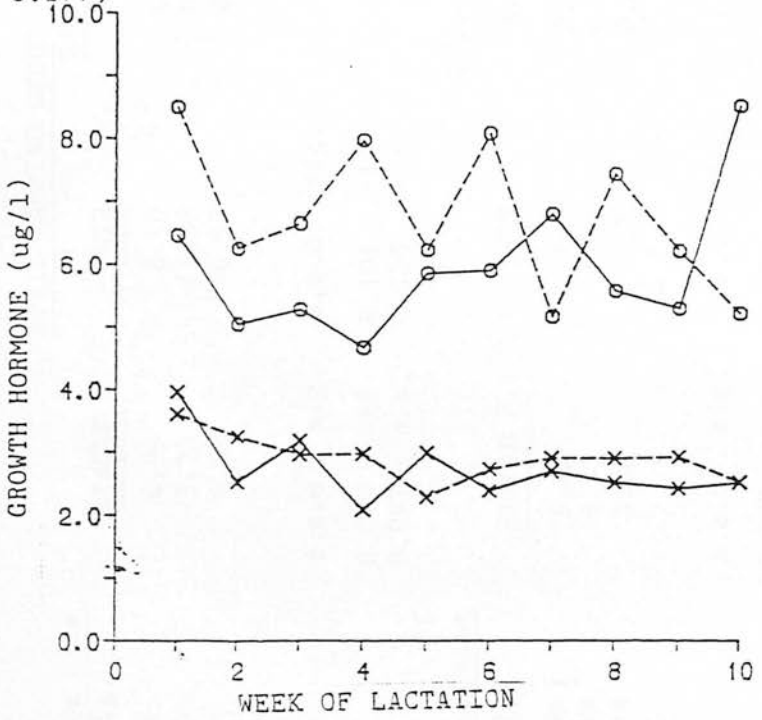


Figure 16. Changes in mean plasma GH concentration (ug/l) during an 8 hour sampling period at week 2 of lactation in S and T ewes at two seasons of year (January: single x——x, twin o——o. April: single x- - -x, twin o- - -o)

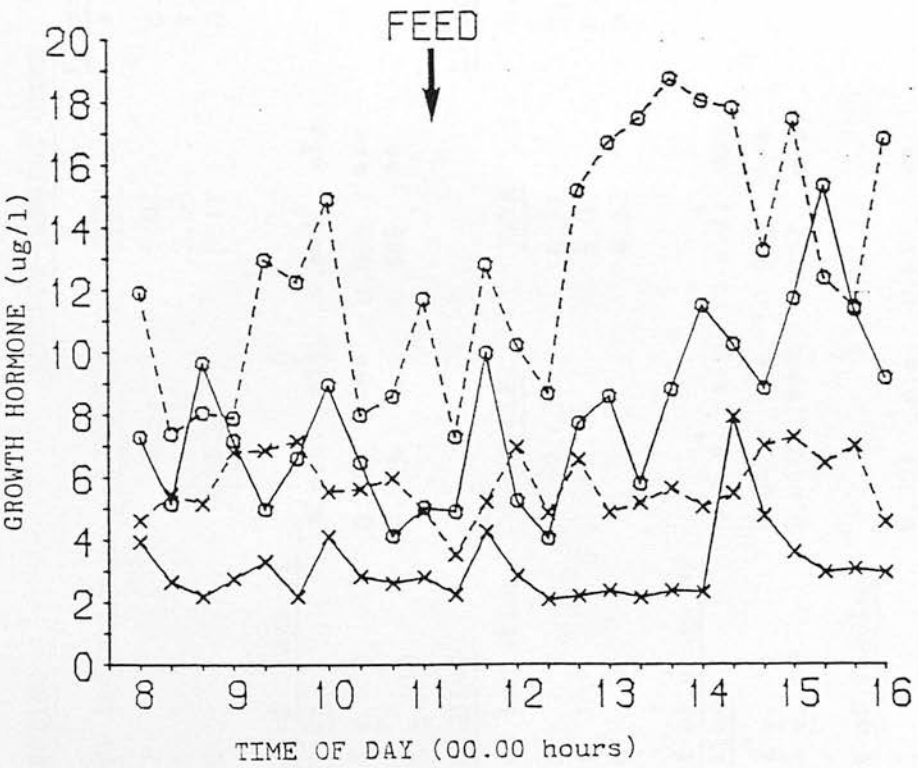


Table 4. Back-transformed overall mean plasma GH concentrations (ug/l) during the pre-feeding (samples 1-9) and post-feeding (samples 13-25) periods in S and T ewes during weeks 2, 4 and 10 of lactation at two seasons of year (January and April).

PRE-FEEDING	JANUARY-LAMBING GROUP				APRIL-LAMBING GROUP			
			Effect of litter size				Effect of litter size	
Week of lactation	SINGLE	TWIN	s.e.d†	sig.	SINGLE	TWIN	s.e.d†	sig.
2	2.53	5.07	0.180	**	4.99	8.10	0.184	*
4	1.77	3.59	1.544	**	3.25	11.88	0.266	***
10	1.93	5.11	0.231	**	4.02	6.57	0.213	n.s.

Effect of stage of lactation	s.e.d.+	sig.	s.e.d.+	sig.	s.e.d.+	sig.	s.e.d.+	sig.
Week 2 v. 4	0.051	***	0.078	***	0.079	***	0.101	***
Week 4 v. 10	0.054	n.s.	0.089	**	0.092	n.s.	0.123	***

POST-FEEDING								
			Effect of litter size				Effect of litter size	
Week of lactation	SINGLE	TWIN	s.e.d.+	sig.	SINGLE	TWIN	s.e.d.+	sig.
2	2.60	6.71	0.184	***	5.22	13.38	0.101	***
4	2.44	5.01	0.108	***	5.33	13.50	0.224	**
10	1.55	4.52	0.189	***	3.46	9.32	0.201	**

Effect of stage of lactation	s.e.d.+	sig.	s.e.d.+	sig.	s.e.d.+	sig.	s.e.d.+	sig.
Week 2 v. 4	0.047	n.s.	0.063	***	0.072	n.s.	0.090	n.s.
Week 4 v. 10	0.040	***	0.067	n.s.	0.077	***	0.101	***
Effect of feeding								
Week 2	0.051	n.s.	0.076	**	0.056	n.s.	0.076	***
Week 4	0.043	***	0.078	***	0.098	***	0.101	n.s.
Week 10	0.048	**	0.068	n.s.	0.072	n.s.	0.076	n.s.

+s.e.d. expressed in log (value+1) units

In general overall mean GH concentration was higher following feeding, although the statistical significance of these differences was independent of litter size or stage of lactation.

Cortisol

Weekly pooled samples:

Mean plasma cortisol concentration tended to increase during early lactation and decrease during late lactation in S and T ewes in each lambing group, although between week variation was large (Figure 17).

Litter size had a highly significant effect on overall mean cortisol concentration in each lambing season. S ewes had a lower value than T ewes in the January-lambing group (4.00 v. 7.41 ug/l; s.e.d. (expressed in log (value+1) units) = 0.132; $P < 0.001$) and the April-lambing group (3.31 v. 6.61 ug/l; s.e.d. (expressed in log (value+1) units) = 0.129; $P < 0.001$). In the individual weeks tested the value in S ewes was significantly lower compared with T ewes during weeks 2 ($P < 0.01$), 4 ($P < 0.05$) and 10 ($P < 0.001$) of lactation in the January group, although during week 2 ($P < 0.001$) of lactation only in the April-lambing group.

There was no effect of season of lambing on overall mean cortisol concentration in either S or T ewes.

Frequently collected samples:

In general plasma cortisol concentration increased steadily prior to feeding, fluctuated markedly around the time of feeding and decreased during the remainder of the sampling period in S and T ewes in each lambing group at each of the three periods (shown for week 2 of lactation in Figure 18).

Overall mean concentration was consistently lower in S ewes compared with T ewes on all occasions in each lambing season although the level of significance of this difference was independent of stage of lactation and feeding status (Table 5).

Figure 17. Back-transformed plasma cortisol concentrations (ug/l) during lactation in S and T ewes at two seasons of year (January: single x——x, twin o——o; s.e.d. (expressed in log (value + 1) units) weeks 2, 4 and 10 = 0.172, 0.225, 0.160. April: single x– – –x, twin o– – –o; s.e.d. (expressed in log (value + 1) units) weeks 2, 4 and 10 = 0.210, 0.214, 0.231)

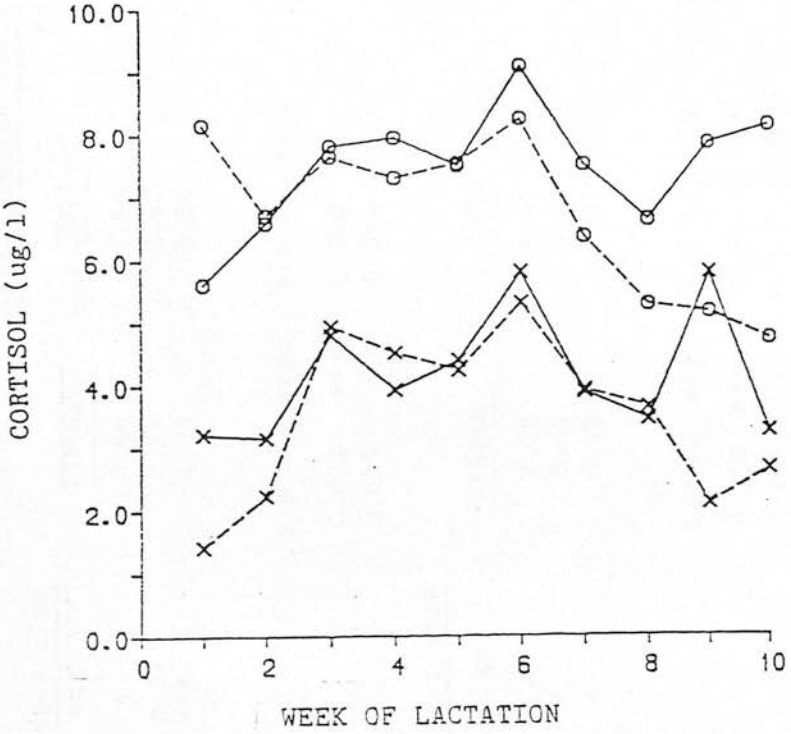


Figure 18. Changes in mean plasma cortisol conetration (ug/l) during an 8 hour sampling period at week two of lactation in S and T ewes at 2 seasons of year (January: single x——x, twin o——o. April: single x– – –x, twin o– – –o)

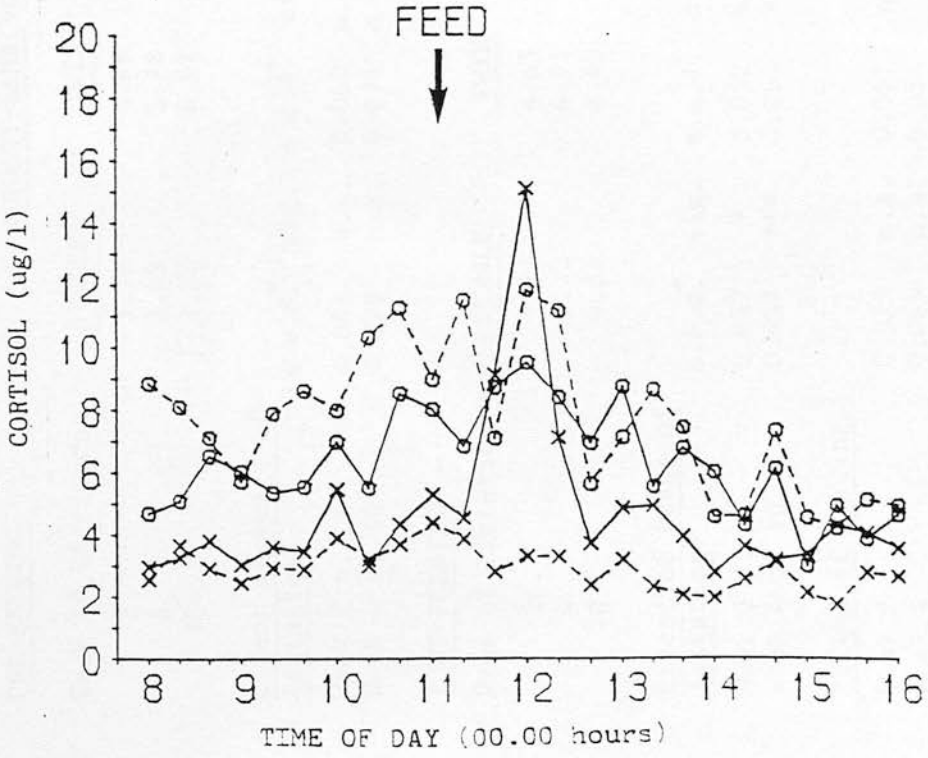


Table 5. Back-transformed overall mean plasma cortisol concentrations (ug/l) during the pre-feeding (samples 1-9) and post-feeding (samples 13-25) periods in S and T ewes during weeks 2, 4 and 10 of lactation at two seasons of year (January and April).

PRE-FEEDING	JANUARY-LAMBING GROUP				APRIL-LAMBING GROUP			
			Effect of litter size				Effect of litter size	
Week of lactation	SINGLE	TWIN	s.e.d†	sig.	SINGLE	TWIN	s.e.d†	sig.
2	3.18	4.99	0.165	*	2.66	7.64	0.167	***
4	3.69	5.76	0.209	n.s.	5.86	7.62	0.128	n.s.
10	3.28	6.82	0.136	***	4.93	6.86	0.121	*

Effect of stage of lactation	s.e.d.+	sig.	s.e.d.+	sig.	s.e.d.+	sig.	s.e.d.+	sig.
Week 2 v. 4	0.091	n.s.	0.069	n.s.	0.071	***	0.060	n.s.
Week 4 v. 10	0.079	n.s.	0.074	n.s.	0.059	*	0.054	n.s.

POST-FEEDING			Effect of litter size				Effect of litter size	
Week of lactation	SINGLE	TWIN	s.e.d.+	sig.	SINGLE	TWIN	s.e.d.+	sig.
2	3.83	4.65	0.139	n.s.	2.25	5.65	0.124	***
4	3.22	4.91	0.148	*	6.43	8.19	0.111	n.s.
10	4.51	6.49	0.140	*	6.98	7.31	0.126	n.s.

Effect of stage of lactation	s.e.d.+	sig.	s.e.d.+	sig.	s.e.d.+	sig.	s.e.d.+	sig.
Week 2 v. 4	0.057	*	0.052	n.s.	0.054	***	0.065	***
Week 4 v. 10	0.057	***	0.063	***	0.058	n.s.	0.078	n.s.
Effect of feeding								
Week 2	0.075	n.s.	0.067	n.s.	0.047	*	0.069	***
Week 4	0.065	n.s.	0.063	*	0.068	n.s.	0.063	n.s.
Week 10	0.072	***	0.063	n.s.	0.070	***	0.071	n.s.

+s.e.d. expressed in log (value+1) units

Changes in overall mean concentration with stage of lactation were not consistent, although levels were generally lower during early lactation (week 2) compared with the two later stages of lactation (weeks 4 and 10).

There was no consistent effect of feeding on overall mean cortisol level.

Prolactin

Weekly pooled samples:

In January-lambing ewes mean plasma prolactin concentration declined during early lactation, remained fairly constant during mid-lactation and increased sharply at the end of the measurement period (Figure 19). In the April-lambing ewes there was no consistent pattern of prolactin concentration in either S or T ewes owing to the erratic nature of the changes in concentration (Figure 19).

There was no difference with litter size in overall mean concentration in each lambing season. In January-lambing ewes values were lower in S than in T ewes during early lactation, although the difference was not significant during week 2 or 4 of lactation. In contrast, values were consistently higher in S ewes than in T ewes during late lactation, in April lambing ewes, and the difference was significant during week 10 ($P < 0.05$) of lactation.

Season of lambing had a highly significant effect on overall mean prolactin concentration. January-lambing ewes had a lower value than April-lambing ewes in S ewes (68.0 v. 296.2 ug/l; s.e.d. (expressed in log units) = 0.17; $P < 0.001$) and T ewes (79.8 v. 240.8 ug/l; s.e.d. (expressed in log units) = 0.22; $P < 0.001$). In the tested weeks the value in January ewes was significantly lower than in April ewes during weeks 2, 4 ($P < 0.001$) and 10 ($P < 0.01$) of lactation in S ewes and similarly weeks 2, 4 ($P < 0.01$) and 10 ($P < 0.05$) of lactation in T ewes.

Figure 19. Back-transformed mean plasma prolactin concentrations (ug/l) during lactation in S and T ewes at two seasons of year (January: single x——x, twin o——o; overall s.e.d. (expressed in log units) = 0.163. April: single x– –x, twin o– –o; overall s.e.d. (expressed in log units) = 0.226)

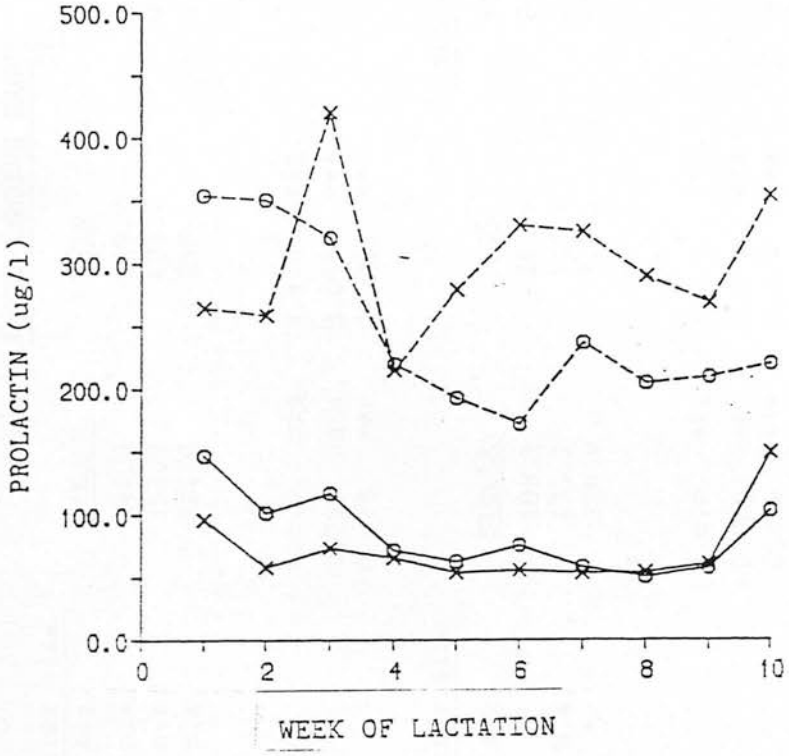


Figure 20. Changes in mean plasma prolactin concentrations (ug/l) during an 8 hour sampling period at week 2 of lactation in S and T ewes at two seasons of year (January: single x——x, twin o——o. April: single x– –x, twin o– –o)

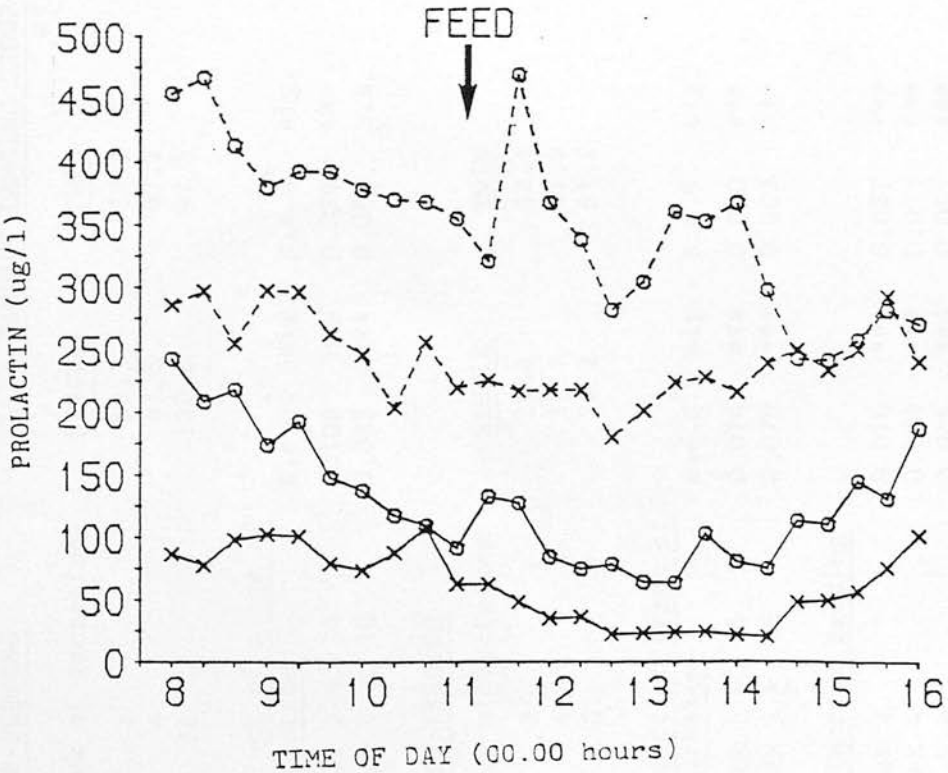


Table 6. Back-transformed overall mean plasma prolactin concentrations (ug/l) during the pre-feeding (samples 1-9) and post-feeding (samples 13-25) periods in S and T ewes during weeks 2, 4 and 10 of lactation at two seasons of year (January and April).

PRE-FEEDING	JANUARY-LAMBING GROUP				APRIL-LAMBING GROUP			
			Effect of litter size				Effect of litter size	
Week of lactation	SINGLE	TWIN	s.e.d+	sig.	SINGLE	TWIN	s.e.d+	sig.
2	61.3	123.3	0.348	n.s.	244.9	349.0	0.202	n.s.
4	67.6	83.2	0.204	n.s.	253.9	207.1	0.207	n.s.
10	140.8	97.5	0.218	n.s.	321.8	258.0	0.140	n.s.

Effect of stage of lactation	s.e.d.+	sig.	s.e.d.+	sig.	s.e.d.+	sig.	s.e.d.+	sig.
Week 2 v. 4	0.106	n.s.	0.079	***	0.057	n.s.	0.059	***
Week 4 v. 10	0.083	***	0.086	n.s.	0.058	***	0.080	**

POST-FEEDING			Effect of litter size				Effect of litter size	
Week of lactation	SINGLE	TWIN	s.e.d.+	sig.	SINGLE	TWIN	s.e.d.+	sig.
2	27.4	62.1	0.312	*	208.5	265.9	0.179	n.s.
4	39.1	41.0	0.197	n.s.	175.7	137.1	0.257	n.s.
10	78.3	61.8	0.170	*	278.9	276.4	0.104	n.s.

Effect of stage of lactation	s.e.d.+	sig.	s.e.d.+	sig.	s.e.d.+	sig.	s.e.d.+	sig.
Week 2 v. 4	0.074	***	0.070	***	0.065	**	0.071	***
Week 4 v. 10	0.079	***	0.065	***	0.066	***	0.074	***
Effect of feeding								
Week 2	0.010	***	0.087	***	0.049	***	0.057	***
Week 4	0.074	***	0.071	***	0.060	***	0.061	***
Week 10	0.046	***	0.061	***	0.062	*	0.069	n.s.

+s.e.d. expressed in log units

Frequently collected samples:

Plasma prolactin concentration tended to decrease during the first 5 hours of the sampling period and then either increased steadily or remained fairly constant throughout the remainder of the sampling period at each of the three periods (shown at week 2 of lactation in Figure 20).

In general there was no significant difference with litter size in overall mean concentration during either the pre- or post-feeding period at each lambing season (Table 6), although there was a marked trend towards lower levels in S compared with T ewes during early lactation (week 2).

Values generally decreased significantly during early lactation (i.e. between week 2 and 4) and increased significantly during later lactation (i.e. between week 4 and 10).

Post-feeding levels were consistently and significantly lower than pre-feeding values on almost all occasions in S and T ewes at each lambing season.

Triiodothyronine (T3)

Weekly pooled samples:

Plasma triiodothyronine concentration did not vary markedly during lactation in S and T ewes at each lambing season (Figure 21).

There was no difference in overall mean concentration with litter size in the January-lambing group. However, the value was significantly higher in S ewes than T ewes (1.37 v. 1.12 ug/l; s.e.d. = 0.129; $P < 0.05$) in the April-lambing group. Individual weekly values were higher in S ewes than in T ewes, differences being significant ($P < 0.05$) in 2 out of the 3 tested weeks (i.e. weeks 4 and 10 of lactation).

Season of lambing had a significant effect on overall mean concentration. January-lambing ewes had a higher value than April-

lambing ewes in both S ewes (1.71 v. 1.37 ug/l; s.e.d. = 0.065; $P < 0.001$) and T ewes (1.71 v. 1.12 ug/l; s.e.d. = 0.134; $P < 0.001$). Values in January-lambing ewes were higher than in April-lambing ewes during weeks 2 and 4 of lactation in S ($P < 0.01$) and T ewes ($P < 0.001$).

Thyroxine (T4)

Weekly pooled samples:

After declining initially, there was an increase in mean thyroxine concentration which was sustained in January-lambing ewes but was more transient in the April-lambing group (Figure 22).

There was no significant difference with litter size in overall mean T^4 concentration at each lambing season. In the April-lambing group values were consistently higher in S ewes compared with T ewes, although there were no significant differences in any of the individual weeks tested.

Season of lambing had no effect on overall mean concentration in either S or T ewes, although in S ewes values tended to be higher in January-lambing than in April-lambing ewes during late lactation and in T ewes values were consistently higher in January-lambing ewes compared with April ewes throughout the whole 10 week lactation period.

Figure 21. Mean plasma T^3 concentrations (ug/l) during lactation in S and T ewes at two seasons of year (January: single x——x, twin o——o; overall s.e.d. = 0.103. April: single x– – –x, twin o– – –o; s.e.d. weeks 2, 4 and 10 = 0.120, 0.123, 0.139)

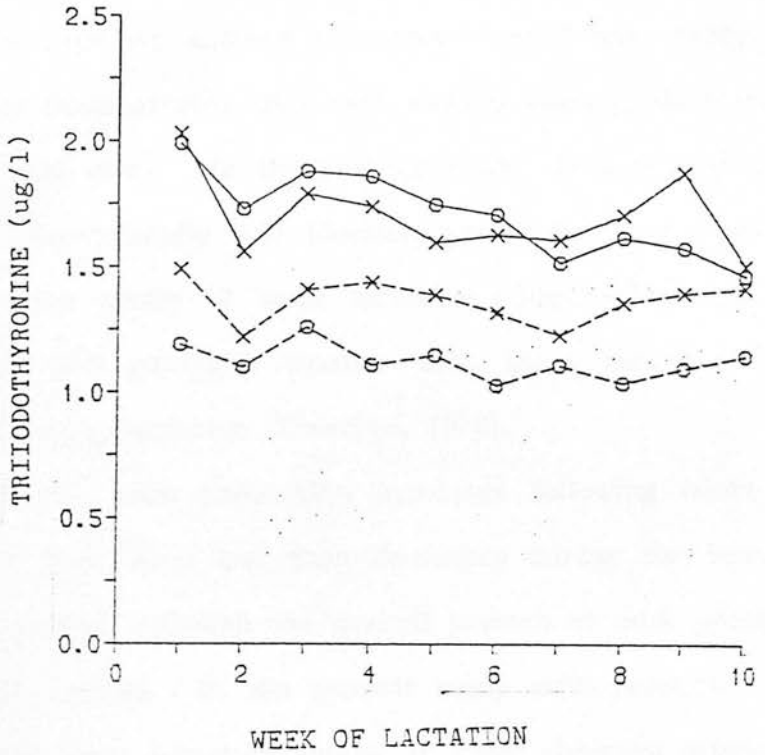
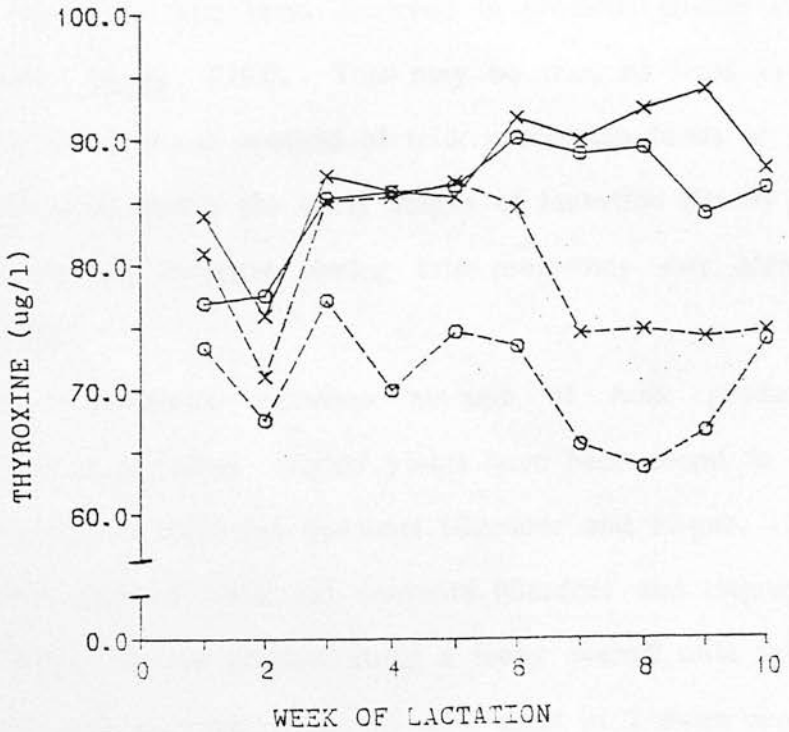


Figure 22. Mean plasma T^4 concentrations (ug/l) during lactation in S and T ewes at two seasons of year (January: single x x, twin o o; overall s.e.d. = 7.12. April: single x x; twin o o; overall s.e.d. = 5.88)



DISCUSSION

MILK PRODUCTION

Many previous authors (Alexander and Davis, 1959; Peart et al., 1972) have demonstrated that twin-suckled ewes produce more milk than single-suckled ewes. In the present study, twin-suckled ewes (T ewes) produced proportionally 0.21 (January group) and 0.17 (April group) more milk over the whole 10 week lactation compared with S ewes. These differences are generally smaller than those reported for ewes well nourished during lactation (Treacher, 1978).

Typically, milk production increases following onset of lactation, reaches a peak level and then decreases during the remainder of the lactation period, although the overall pattern of milk production depends on various factors. In the present study milk production profiles in S and T ewes were generally similar to those observed previously (Peart et al 1972; Peart, Edwards and Donaldson, 1975), except there was no increase in milk yield during early lactation, milk production being maximum during the first week of lactation in each rearing group. A similar result has also been observed in previous studies (Doney et al., 1979, Doney et al., 1983). This may be due, at least in part, to the fact that the oxytocin method of milk extraction tends to over-estimate milk production during the early stages of lactation (Doney et al., 1979). A high level of nutrition during late pregnancy may also be involved (Peart, 1982).

The relationship between amount of milk produced and its composition is variable. Higher yields have been found to be associated both with higher milk fat contents (Gardner and Hogue, 1964; Peart et al., 1972) and lower milk fat contents (Gardner and Hogue, 1966; Peart et al., 1979). In the present study a lower overall milk fat content was associated with the higher overall milk yield in T ewes compared with S ewes.

The results of the present study with respect to milk protein, lactose and ash contents also agree with those of Gardner and Hogue (1964) and Peart et al. (1972) who demonstrated that there was no difference in overall mean values of any of these constituents associated with the higher milk yield in twin-suckled compared with single-suckled ewes.

In general, changes in milk composition during lactation were similar to those previously reported (Gardner and Hogue, 1964; Peart et al., 1972), although in contrast to the previous reports, in the present study milk protein content tended to decrease while milk lactose content continued to increase slightly during the later stages of the 10 week lactation study.

The extensive literature on milk production in sheep shows that differences in amount, pattern and composition can be expected in relation to differences in suckling demand. The objective, achieved in the present study, was to create these differences in both the January- and April-lambing groups so that associated differences in circulating hormone and blood metabolite status could be examined.

NUTRITIONAL STATUS

One of the most important factors limiting milk production is the supply of nutrients to the mammary gland. Understanding of the processes which result in different milk yields requires assessment of the supply of nutrients available for milk synthesis. During early lactation these nutrients are usually derived from both ingested food and body tissue. In the present study dietary nutrient supply was similar for ewes rearing either single or twin lambs as ewes in each group consumed a fixed amount of feed. It was assumed, therefore, that treatment differences in nutrient requirements for milk production would be reflected in differences in the degree of utilisation of body reserves.

In order to assess the relative importance of the supply of nutrients derived from tissue mobilisation, changes in live weight, body condition score and levels of several blood metabolites associated with energy and protein status were measured throughout lactation.

In the present study erratic changes in live weight and body condition make the interpretation of overall changes difficult. There are several problems associated with the use of these parameters as indicators of body tissue metabolism. For example fat loss is accompanied by an increase in body water content (Foot, Skedd and McFarlane, 1979; Cowan et al., 1980). This may explain, at least in part, the erratic nature of live weight changes throughout lactation, particularly when considered in conjunction with differences in condition score at lambing.

Notwithstanding these limitations it is clear while S ewes generally maintained or increased their live weight and body condition score throughout lactation, T ewes generally lost live weight and body condition score. These trends are consistent with a greater degree of mobilisation of body tissue when ewes are suckling twin compared with single lambs.

Energy status

Circulating levels of glucose, non-esterified fatty acids (NEFA) and ketone bodies all constitute indices of energy status. Lactating animals have a specific requirement for glucose as it is the principle nutrient precursor for lactose production and it has been suggested that glucose supply to the mammary gland may be a limiting factor for milk production (Kronfield, 1976).

Glucose concentrations are generally a poor index of energy status, probably because of the strict homeostatic control to which they are subject. Nevertheless glucose flow rate is related to circulating glucose

levels (Lindsay, 1978) and it was felt that estimates of plasma glucose levels may provide some indication of glucose turnover in ewes.

The absence of any difference in glucose concentration between S and T ewes, despite the fact that glucose requirements for lactose production would be expected to be increased in T ewes compared with S ewes, illustrates the difficulties in interpretation of glucose levels. The general increase in glucose levels during the course of lactation is consistent with the decrease in glucose requirement of the mammary gland in each of the rearing groups.

Circulating NEFA concentrations are related to energy status and rate of adipose tissue mobilisation (Annison 1960; Reid and Hinks, 1962a and b; Vernon, 1980). Levels of approximately 450 mM/l have been recorded in sheep fed according to maintenance requirements (Russel and Doney, 1969), and of between 1500 and 2500 mM/l in starved sheep (Annison, 1960). The elevated NEFA levels during early lactation in both S and T ewes indicate that ewes were mobilising adipose tissue during this period and the significantly higher values in T ewes compared with S ewes suggests that T ewes were utilising adipose tissue at a greater rate than S ewes. This is consistent with results of Vernon et al. (1981), and also with the general trends in live weight and body condition changes observed in this experiment.

However, it seems unlikely that the increase in NEFA concentration in S ewes during mid-lactation is attributable to an increase in the rate of adipose tissue mobilisation, particularly in view of the fact that the April-lambing ewes were gaining live weight during this period. A small proportion of circulating NEFA is derived from the diet by direct absorption in the small intestine and some is also released from the mammary gland (Annison, Linzell, Fazakerley and Nichols, 1967), possibly as a result of the hydrolysis of triglyceride in the mammary gland (West,

Bickerstaffe, Annison and Linzell, 1972). In the absence of information on the proportion of NEFA derived from each of these sources it is not possible to draw further conclusions with regard to differences in patterns of circulating NEFA levels in S and T ewes. Furthermore, circulating levels are a function of input, rate of turnover and pool size, all of which must be considered when interpreting data of this nature.

The third index of energy status measured was 3-hydroxybutyrate (3-OHB) which is a ketone body derived from the oxidation of NEFA under conditions of energy deficit. It is generally regarded as a more appropriate index of energy status than circulating NEFA levels in cases of more severe under-nutrition (Russel et al., 1967; Bowden, 1971). Furthermore, unlike circulating glucose and NEFA levels, 3-OHB levels are not influenced to the same extent by stressful stimuli e.g. restraint and blood sampling (Russel, 1978). In a recent study, 3-OHB concentrations have been found to be positively correlated with the number of lambs suckled, in agreement with the present study, and growth of lambs (Foot, Cummins, Spiker and Flinn, 1985).

The higher 3-OHB levels in T compared with S ewes reflects the greater degree of energy deficit in T ewes compared with S ewes during early lactation, which is consistent with the increase in milk yield in T ewes compared with S ewes during this period. Although 3-OHB is derived from the oxidation of butyrate in the rumen epithelium and liver, as well as from oxidation of NEFA, it is unlikely that the marked differences in 3-OHB concentration between rearing groups were attributable to difference in 3-OHB production via this mechanism as food intakes were similar in both rearing groups.

Protein status

Circulating urea concentration is a short-term index of protein status, while albumin and total protein can both be considered as indices

of long-term protein status. However, like indices of energy status, these parameters are limited in their usefulness. For example circulating urea concentration can be elevated under both conditions of dietary protein sufficiency or insufficiency because it is a product of both dietary amino acid deamination and of body protein degradation (Sykes, 1978). Circulating albumin and total protein concentrations are, arguably, more useful estimates of long-term protein status as their levels are likely to be reduced under conditions of protein deficiency (Sykes, 1978).

In view of the relationship between urea entry and excretion rates and plasma urea concentration (Sykes, 1978) it might have been expected that circulating urea concentration would have been lower in T ewes than in S ewes, as milk protein production was higher in the former group and thus urea nitrogen excretion would be expected to be reduced. However, plasma urea concentrations were in fact similar in each of the rearing groups throughout lactation. An alternative explanation is that urea levels were increased in T ewes as a result of mobilisation of muscle protein in order to obtain increased supplies of amino acid for milk protein synthesis or gluconeogenesis. It is unlikely, however, that mobilisation of protein stores occured during the whole of the lactation period in T ewes. Another possibility is that urea levels recorded in the present study are not closely related to urea entry and excretion rates as this has shown to be the case within a limited range of plasma urea levels (McIntyre, 1970).

Similarities in plasma albumin and total protein concentration between rearing groups suggests that there were no differences in long-term protein status between S and T ewes during lactation. The increase in albumin concentration observed in all treatment groups is probably attributable to an increase in amino acid availability as amino acid requirements for milk production decrease.

While blood metabolites are clearly not ideal estimators of energy and protein status, the results of the present study demonstrate differences in some of the indices of energy status which are consistent with differences in milk production between rearing groups. Taken together the indices of nutritional status suggest that the differences in milk production, at the given level of feed intake were associated with differences in energy although not protein status. However, it must also be remembered that energy and protein metabolism pathways are inextricably linked and as a result should not be considered separately (Oldham, 1984).

It is possible that differences in blood metabolite levels associated with nutritional status constitute part of the driving force behind endocrine changes which may also be involved in induction of differences in milk production. Both induction of changes in metabolite level and the effects of such changes are mediated through hormonal factors.

HORMONE STATUS

Increased milk production in T ewes compared with S ewes was associated with a lower weekly insulin concentration and a higher weekly growth hormone (GH) concentration. This is consistent with the increased requirement for energy substrates for milk production in T ewes compared with S ewes and the known anabolic and catabolic roles of these hormones (Bassett, 1978; Trenkle, 1981). It is also consistent with the results of Hart et al. (1975; 1978) who found that high-yielding dairy cows had lower insulin and higher GH levels than low-yielding cows.

In view of the opposite effects of these hormones on adipose tissue metabolism (Trenkle, 1981) the observed relationship between insulin and GH in T ewes compared with S ewes would be expected to result in a greater degree of tissue mobilisation and a lesser degree of nutrient

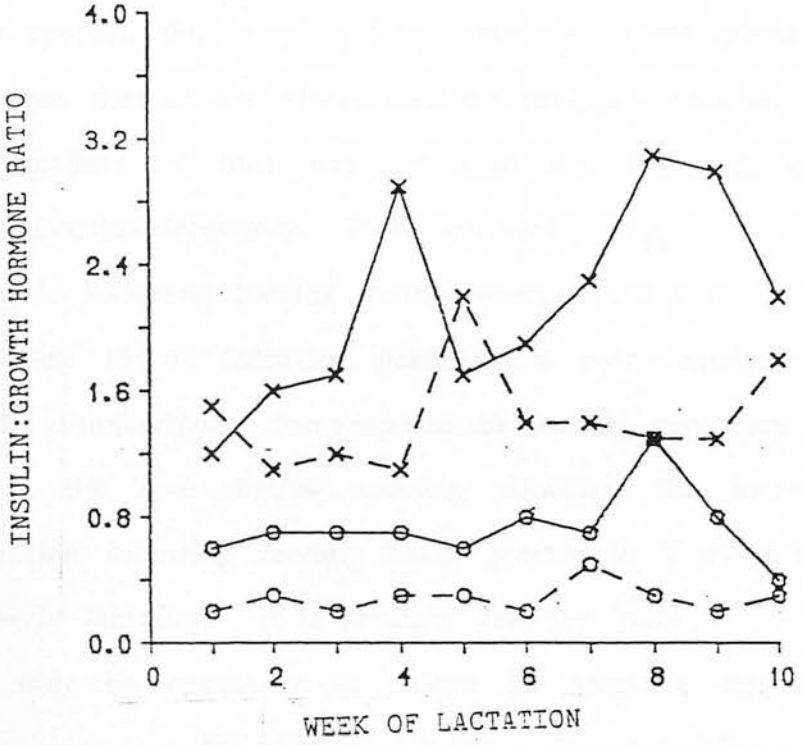
anabolism in T ewes during early lactation and thus greater nutrient availability for milk production (Figure 22a). It is difficult, however, to reconcile the observed differences in hormone concentration in late lactation with estimates of energy status (NEFA and 3-OHB levels) and milk production, both of which were similar in the two rearing groups at this stage. Furthermore, there were no marked changes in weekly insulin or GH level associated with stage of lactation, which might be expected in view of the large changes in the rate of adipose tissue mobilisation and milk production taking place during lactation.

It should be noted that when interpreting circulating hormone concentrations, the radioimmunoassay technique does not necessarily estimate the biological activity of the measured hormones. In addition hormone action is mediated by a complex system of receptor sites and feed-back mechanisms (Cowie et al., 1980). In the present study, it is possible that additional unidentified factors are involved in the control of metabolic processes. This could explain why differences in insulin and GH concentration and the ratio of these hormones between rearing groups during the latter half of lactation were not apparently associated with differences in milk production and associated nutrient metabolism.

Although analysis of pooled weekly samples provides an overall assessment of endocrine status throughout lactation, several hormones are increased in the short-term in response to an increase in the supply of nutrients in the blood stream following feeding (Trenkle, 1978; Weekes and Godden, 1981). Blood sample collection throughout the day at selected stages of lactation permitted assessment of the short-term endocrine responses following feeding at different stages of lactation in each rearing groups.

While the marked post prandial increase in insulin concentration was considered normal (Bassett, 1974a, b), the rise in the GH

Figure 22a. Mean plasma insulin : geometric GH ratio during lactation in S and T ewes at 2 seasons of year (January: single x——x, twin o——o; April: single x- -x, twin o- -o)



concentration following feeding in some groups contrasts with previous findings from work using wethers (Trenkle, 1971) and lactating cattle (Bines et al., 1983). In addition to differences in either physiological state or species, the sampling frequency used in the present study was greater than that of the aforementioned studies. Thus differences may have been detected that may not have been apparent with a lesser sample collection frequency. It is noteworthy that the mean GH levels were higher following feeding during weeks 2 and 4 of lactation, while during week 10 of lactation generally a post prandial decline was recorded. Furthermore, the response to feeding may vary with energy status of the ewe and/or suckling stimulus, the increase in GH concentration following feeding being greater in T ewes than S ewes during early lactation. It is possible that the increase in GH following feeding may be necessary to ensure an adequate supply of energy providing substrates for milk production. The mechanism by which GH operates, however, is not clear.

The absence of any consistent changes in the average pre- and post-feeding insulin and GH levels with advancing stage of lactation is consistent with results obtained from the pooled weekly samples. However, the trend towards lower GH concentrations particularly following feeding, by week 10 of lactation suggests that post prandial levels of this hormone were modified according to the stage of lactation, and the change in nutrient requirements for milk production.

Endocrine status should not be considered solely in relation to energy metabolism. The lower insulin concentration, which was associated with higher milk production in T ewes compared with S ewes, would be expected not only to decrease the accretion of energy providing substrates but also to reduce protein synthesis in peripheral tissues in T ewes more than in S ewes (Trenkle, 1981). This would be

expected to result in an increase in amino acid availability to the mammary gland which is consistent with the increased levels of milk protein production in T ewes compared with S ewes.

Cortisol is also known to influence both energy and protein metabolism (Trenkle, 1981). The higher cortisol concentration in T ewes compared with S ewes is consistent with results of Johnson and Vanjonack (1975) comparing high- and low-yielding dairy cows and with the known stimulatory influence of cortisol on rate of gluconeogenesis in response to increased glucose requirements (McDowell, 1983). However, there was no evidence of a difference in long-term protein status (as measured by plasma albumin and total protein content) between S and T ewes despite the catabolic effect of cortisol on body protein (Trenkle, 1981).

Cortisol has been shown to exhibit a stimulatory influence on insulin (Bassett and Wallace, 1967), but in the present study the higher cortisol concentration in T ewes compared with S ewes was associated with a lower insulin concentration in T ewes throughout lactation. This would favour nutrient partitioning towards the mammary gland and is consistent with the increase in milk yield in T ewes compared with S ewes. The absence of any marked change in this relationship with advancing stage of lactation suggests that the change in this particular hormonal relationship per se is not involved in the switch in priority of supply of nutrients from the mammary gland to body tissue.

The absence of any consistent change in cortisol concentration following feeding confirms the findings of Bassett (1974b) and suggests that cortisol alone is not a major factor in the control of nutrient availability during the postprandial period.

The relationship between cortisol and GH is worthy of consideration in relation to both energy and protein status. The increased levels of

both hormones in high compared with low yielding animals is consistent with the role of both hormones in stimulating supply of energy yielding substrates (Trenkle, 1981; Hart, 1983). In relation to protein status, the known roles of the two hormones oppose each other with cortisol exerting a catabolic influence on body protein while the role of GH is anabolic (Hart, 1983). In lactating animals it is thought that the increased levels of GH may act to increase the uptake of amino acids at the mammary gland (Bauman and McCrutcheon, 1986). The fact that the actions of both GH and cortisol facilitate nutrient partitioning towards the mammary gland in terms of both energy and protein, is consistent with the finding that the levels of both of these hormones were higher in T compared with S ewes.

Superimposed on the effects of hormones which are known to influence maternal metabolism and circulating nutrient concentrations during lactation, there are possible effects of other hormones which may be involved in the control of milk production. Prolactin in known to be involved in the establishment of lactation (Cowie et al., 1980), although its role in maintenance of milk production and nutrient metabolism in ruminant species is questionable. Prolactin concentration is also known to be positively related to increasing daylength and temperature (Lamming et al., 1974). The fact that the higher milk production in T ewes compared with S ewes was not associated with any difference in prolactin concentration suggests that prolactin is not involved in the control of tissue mobilisation and nutrient supply, in contrast to evidence suggesting a role for prolactin in adipose tissue mobilisation in non-ruminant species (Bauman and Currie, 1980). The results of the present study are consistent with those of Hart et al. (1978) who showed that there was no difference in circulating prolactin concentration in high- compared with low-yielding dairy cows.

The absence of any influence of season of lambing on milk yield in conjunction with large seasonal differences in prolactin concentration provides further evidence that prolactin concentration per se is not a limiting factor in the control of milk yield in the present study.

The lack of any apparent response in prolactin values to feeding, although contrasting with previous results (Trenkle, 1978), is probably not of biological significance and may simply reflect diurnal variation in this hormone.

The thyroid hormones (i.e. T4 and the more biologically active T3), which are responsible for the control of metabolic rate in peripheral tissues (Bernal and Refetoff, 1972), have also been implicated in the control of lactation (Fulkerson, 1979; Cowie et al., 1980). The finding that differences in the levels of the thyroid hormones between rearing groups were generally small and non significant, despite differences in milk yield, agrees with previous work comparing high- and low-yielding dairy cows (Hart et al., 1978; Walsh et al., 1980). These results are difficult to reconcile with the marked increase in milk yield observed when T4 levels are stimulated using exogenous T4 administration (Blaxter et al., 1949). This suggests that the mode of T4 action in the control of milk production is complex and that the relatively crude technique of injection of exogenous hormone may not be adequate to provide a complete understanding of the role of this hormone.

Changes in either T3 or T4 concentration with stage of lactation were neither consistent or marked, although the general increase in T4 levels with advancing stage of lactation is consistent with findings of others (Hart et al., 1978; Walsh et al., 1980). Further information is required on the rate at which T4 is converted to T3 and the metabolic clearance rates of these hormones during lactation in ewes of each treatment.

The higher thyroid hormone levels in January- compared with April-lambing ewes may be a function of season (Trenkle, 1978) but the biological significance of the difference remains unclear.

It is possible that the thyroid hormones may act synergistically with other hormones or facilitate their actions in the control of milk production perhaps through actions on receptor site numbers or enzyme activity. However it is clear that much remains to be done to determine the precise actions of the thyroid hormones with respect to the control of milk production.

CONCLUSION

It has been established previously that all the hormones considered in this study are involved in the control of onset and/or maintenance of milk production in the ewe. Hormones and relationships between hormones have been discussed in relation to their known effects on energy and/or protein metabolism. It is quite clear that the overall endocrine control of milk production is multifactorial and highly complex. The results of this study, however, have described some of the relationships between hormones and the way in which these change in relation to differences in milk production and milk composition changes and level of milk production throughout lactation.

Relationships between insulin and GH, cortisol and insulin and GH and cortisol may all be important in relation to nutrient partitioning during lactation in view of their respective influences on nutrient availability. Prolactin does not appear to have a direct role in the control of availability of nutrients during lactation. It is concluded that the thyroid hormones may exert a permissive influence over the action of these and other hormones during lactation.

CHAPTER 5

THE EFFECT OF FEEDING TWO LEVELS OF CRUDE PROTEIN IN THE DIET (EXPT. 3)

RESULTS

MILK PRODUCTION

As in the previous experiments it was not practicable to randomise treatment groups accurately in terms of number of lambs born. Therefore the effect of number of lambs born on milk yield during week 1 of lactation was examined. There was no significant difference in daily milk yield during week 1 of lactation in ewes which bore a single, and suckled twin lambs (mean = 2.92 kg/day; n = 7; s.e. = 0.381) compared with ewes which bore and suckled twin lambs (mean = 2.70 kg/day; n = 8; s.e. = 0.215).

Mean daily milk production increased during early lactation and decreased during mid- and late lactation in each diet group (Figure 23). Peak yield was attained during week 3 of lactation in ewes fed the high protein diet (H ewes) and during week 4 of lactation in ewes fed the low protein diet (L ewes).

There was a significant difference with protein content of the diet in overall mean (i.e. grand mean over the whole 10 week lactation) daily milk yield. L ewes had a lower value than H ewes (2.71 v. 3.10 kg/day; s.e.d. = 0.184; $P < 0.05$). In the 3 individual tested weeks (i.e. weeks 2, 4 or 10 of lactation) differences were not significant.

MILK COMPOSITION

Changes in mean milk fat, protein, lactose and ash content throughout lactation are illustrated in Figures 24 to 27 respectively.

Fat

Maximum mean milk fat content occurred during week 1 of lactation and values generally decreased throughout lactation in each diet group.

Figure 23. Mean daily milk production (kg/day) during lactation in ewes fed either a low x——x or high o——o protein diet (s.e.d. weeks 2, 4 and 10 = 0.320, 0.331, 0.279)

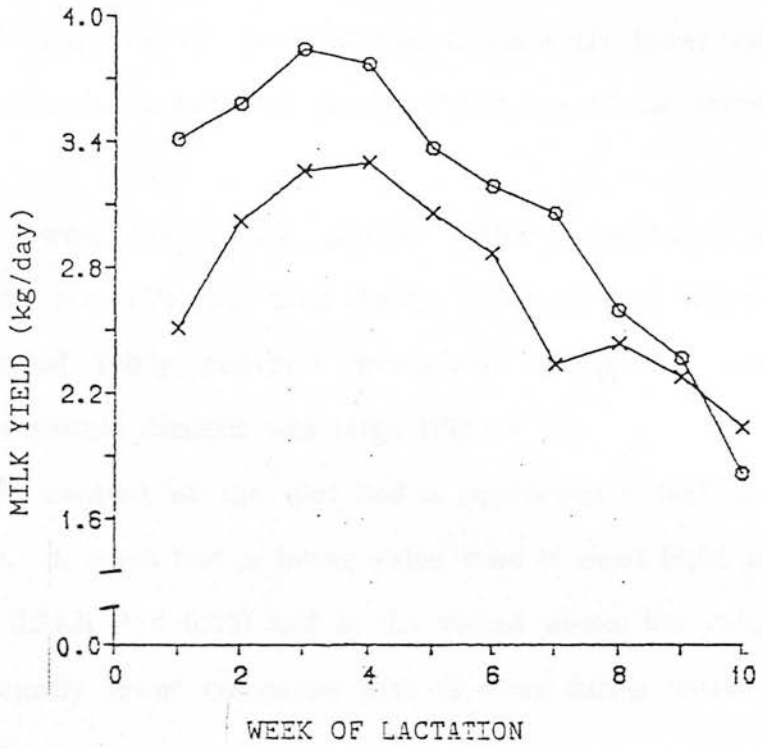
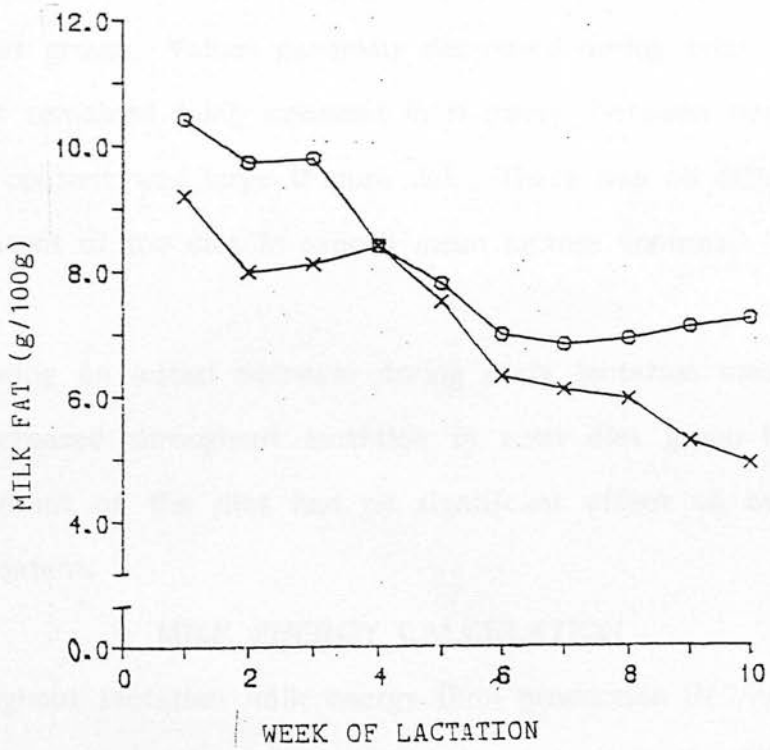


Figure 24. Mean milk fat contents (g/100 g) during lactation in ewes fed either a low x——x or high o——o protein diet (s.e.d. weeks 2, 4 and 10 = 0.846, 0.855, 1.212)



There was no significant effect of protein content in the diet on the overall mean value. L ewes had consistently lower values than H ewes, but differences were not significant in any of the tested weeks.

Protein

In L ewes, mean milk protein content decreased during early lactation and generally increased during late lactation, while values in H ewes remained fairly constant throughout lactation; between week variation in protein content was large (Figure 25).

Protein content of the diet had a significant effect on the overall mean value. L ewes had a lower value than H ewes (6.06 v. 6.60 g/100 g; s.e.d. = 0.242; $P < 0.05$) and in the tested weeks the value in L ewes was significantly lower compared with H ewes during week 2 ($P < 0.05$) of lactation.

Lactose

Mean milk lactose content generally increased during early lactation in each diet group. Values generally decreased during later lactation in L ewes but remained fairly constant in H ewes; between week variation in lactose content was large (Figure 26). There was no difference with protein content of the diet in overall mean lactose content.

Ash

Following an initial decrease during early lactation mean milk ash content increased throughout lactation in each diet group (Figure 27). Protein content of the diet had no significant effect on overall mean milk ash content.

MILK ENERGY CALCULATION

Throughout lactation milk energy (Em) production (MJ/kg DM) in L and H ewes was closely and linearly related to milk fat (Fm) content (g/100 g). The linear regressions were as follows:

Figure 25. Mean milk protein contents (g/100 g) during lactation in ewes fed either a low x——x or high o——o protein diet (s.e.d. weeks 2, 4 and 10 = 0.399, 0.546, 0.463)

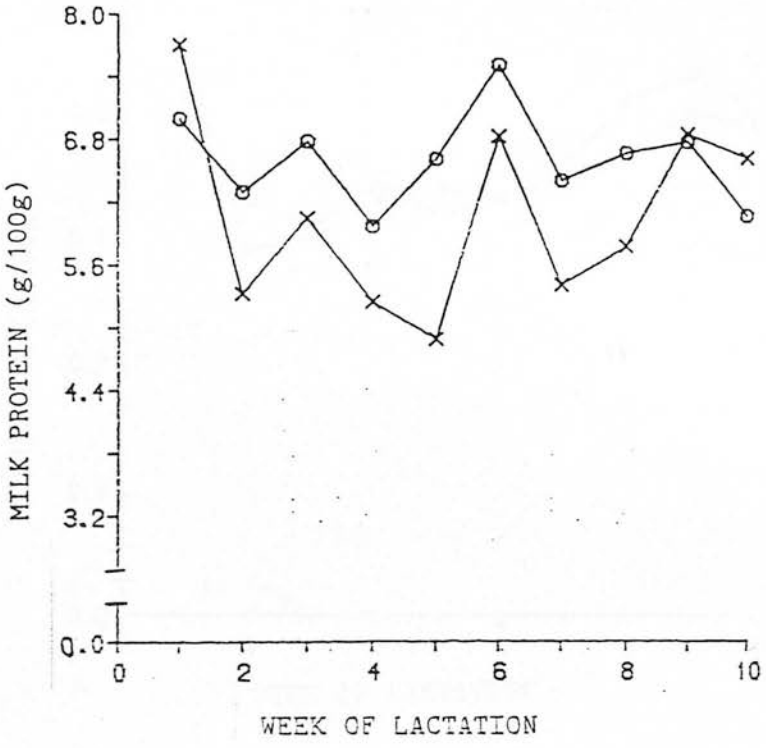


Figure 26. Mean milk lactose contents (g/100 g) during lactation in ewes fed either a low x——x or high o——o protein diet (overall s.e.d. = 0.203)

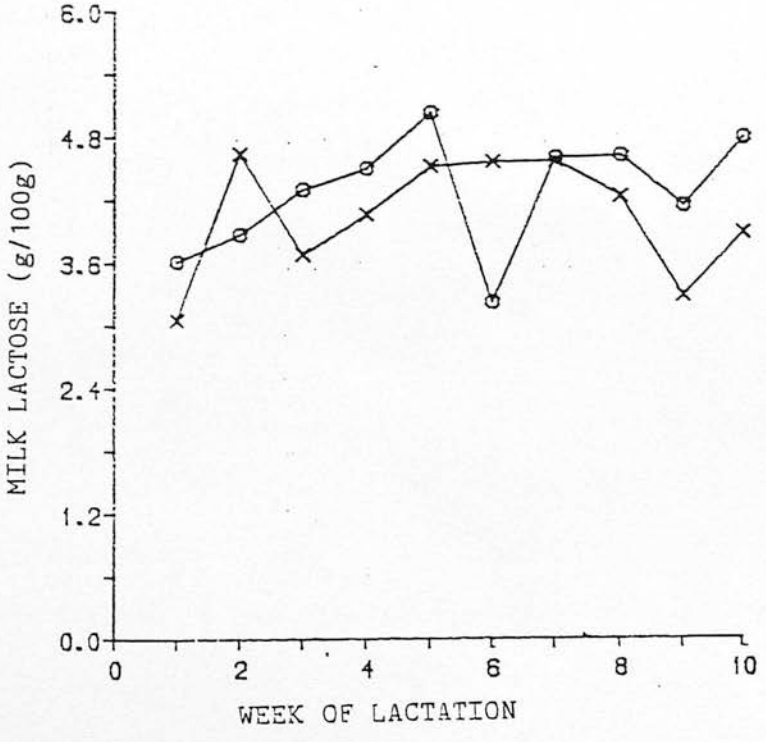
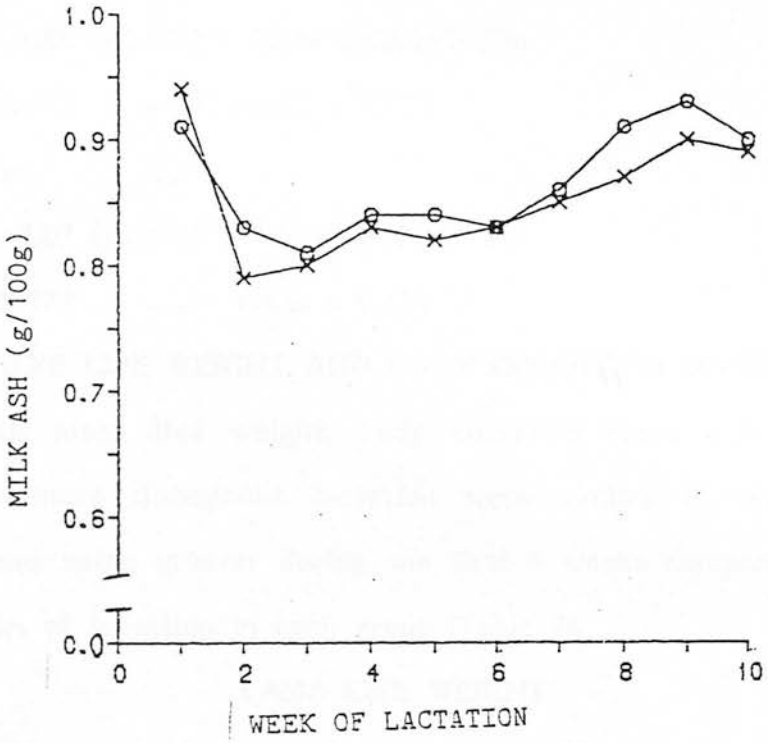


Figure 27. Mean milk ash contents (g/100 g) during lactation in ewes fed either a low x——x or high o——o protein diet (overall s.e.d. = 0.010)



1. L ewes

   $Em = 1.84 (\pm 0.107) + 0.394 (\pm 0.0143) Fm$

   $r^2 = 0.970 \quad n = 25 \quad r.s.d. = 0.139$

2. H ewes

   $Em = 2.07 (\pm 0.111) + 0.389 (\pm 0.0122) Fm$

   $r^2 = 0.977 \quad n = 25 \quad r.s.d. = 0.155$

EWE LIVE WEIGHT AND BODY CONDITION SCORE

Overall mean live weight, body condition score and changes in these parameters throughout lactation were similar in the two diet groups, losses being greater during the first 4 weeks compared with the last 6 weeks of lactation in each group (Table 7).

LAMB LIVE WEIGHT

There was no effect of feeding ewes different levels of dietary protein on mean lamb birth weight, live weight at 10 weeks of age and rate of live weight gain throughout lactation (Table 8).

WEEKLY POOLED BLOOD METABOLITE CONCENTRATIONS

Glucose

Mean plasma glucose concentration tended to increase in early and mid-lactation although declined slightly during late lactation in each diet group (Figure 28). There was no effect of dietary protein content on overall mean glucose concentration.

Non-esterified fatty acids

Mean plasma NEFA concentration increased during early lactation and declined between week 4 and 10 of lactation in each diet group (Figure 29).

Protein content of the diet had no significant effect on overall mean concentration but in L ewes values were consistently higher than in H ewes and in 2 of the 3 individual weeks tested differences were significant, i.e. in weeks 2 ($P < 0.001$) and 10 ($P < 0.05$) of lactation.

Table 7. Mean ewe live weights (kg) and body condition scores postpartum, and at weeks 4 and 10 of lactation

Diet Group	LOW	HIGH	s.e.d.	sig.
Live weight (kg)				
Postpartum	68.5	69.3	2.69	n.s.
Week 4	60.3	61.6	2.37	n.s.
Week 10	60.0	60.1	2.14	n.s.
Body condition score				
Postpartum	2.33	2.33	0.118	n.s.
Week 4	2.06	2.17	0.100	n.s.
Week 10	2.03	2.14	0.110	n.s.

Table 8. Mean lamb live weights (kg) at birth and 10 weeks of age and mean live weight gains (g/day) during 0-4 weeks and 4-10 weeks of lactation.

Diet Group	LOW	HIGH	s.e.d.	sig.
Live weight (kg)				
Birth	5.1	5.0	0.41	n.s.
10 weeks	21.3	21.4	1.01	n.s.
Live weight gain (g/day)				
0-4 weeks	311	319	17.1	n.s.
4-10 weeks	233	231	17.1	n.s.

Figure 28. Mean plasma glucose concentrations (mM/1) during lactation in ewes fed either a low x——x or high o——o protein diet (overall s.e.d. = 0.115)

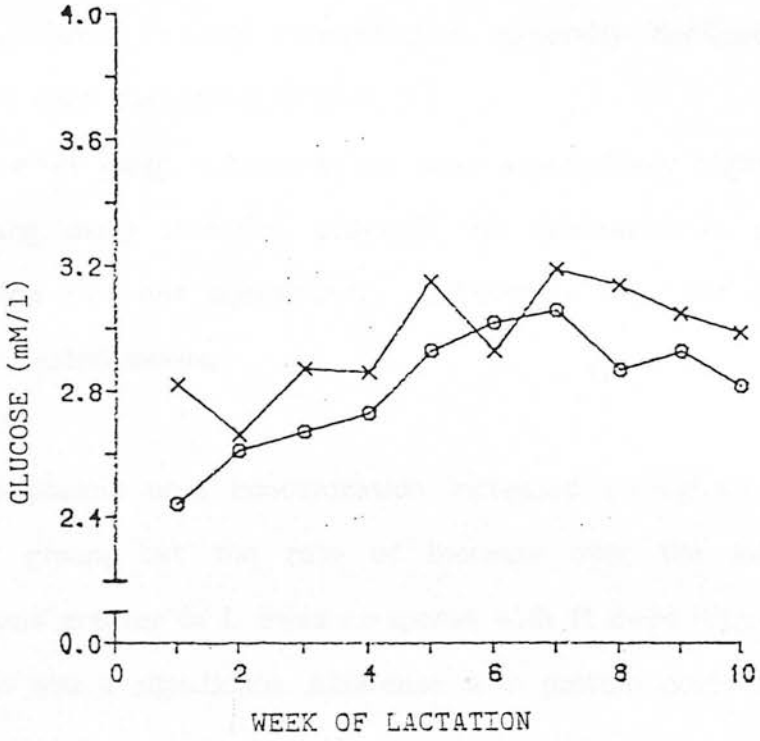
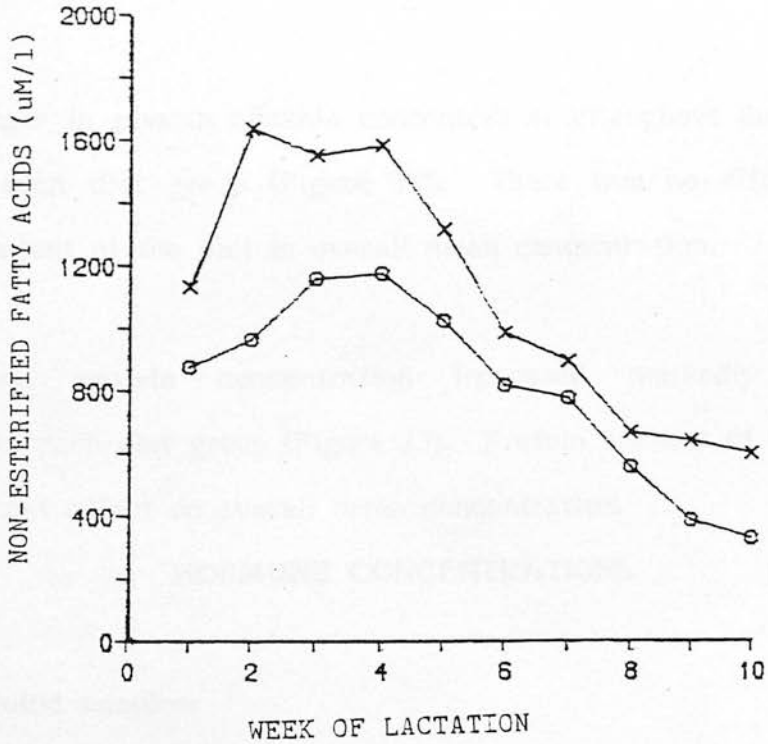


Figure 29. Mean plasma NEFA concentrations (uM/1) during lactation in ewes fed either a low x——x or high o——o protein diet (s.e.d. weeks 2, 4 and 10 = 198.8, 282.6, 142.4)



3-hydroxybutyrate

Mean plasma 3-OHB concentration generally declined throughout lactation in each diet group (Figure 30).

In L ewes mean concentrations were substantially higher than in H ewes, during early lactation although the difference in overall mean concentration was not significant. Differences were not significant at any of the tested weeks.

Urea

Mean plasma urea concentration increased throughout lactation in each diet group, but the rate of increase over the latter half of lactation was greater in L ewes compared with H ewes (Figure 31).

There was a significant difference with protein content of the diet in overall mean concentration. L ewes had a lower value compared with H ewes (5.35 v. 9.61 mM/l; s.e.d. = 0.563; $P < 0.001$). Values in L ewes were lower than in H ewes during 2 of the tested weeks; weeks 2 and 4 ($P < 0.001$) of lactation.

Albumin

Changes in plasma albumin concentration throughout lactation were small in each diet group (Figure 32). There was no difference with protein content of the diet in overall mean concentration.

Protein

Plasma protein concentration increased markedly throughout lactation in each diet group (Figure 33). Protein content of the diet had no significant effect on overall mean concentration.

HORMONE CONCENTRATIONS

Insulin

Weekly pooled samples:

Mean plasma insulin concentration increased throughout most of the 10 week lactation in each diet group (Figure 34).

Figure 30. Back-transformed mean plasma 3-OHB concentrations (mM/1) during lactation in ewes fed either a low x——x or high o——o protein diet (s.e.d. (expressed in log (value + 1) units) weeks 2, 4 and 10 = 0.157, 0.130, 0.034)

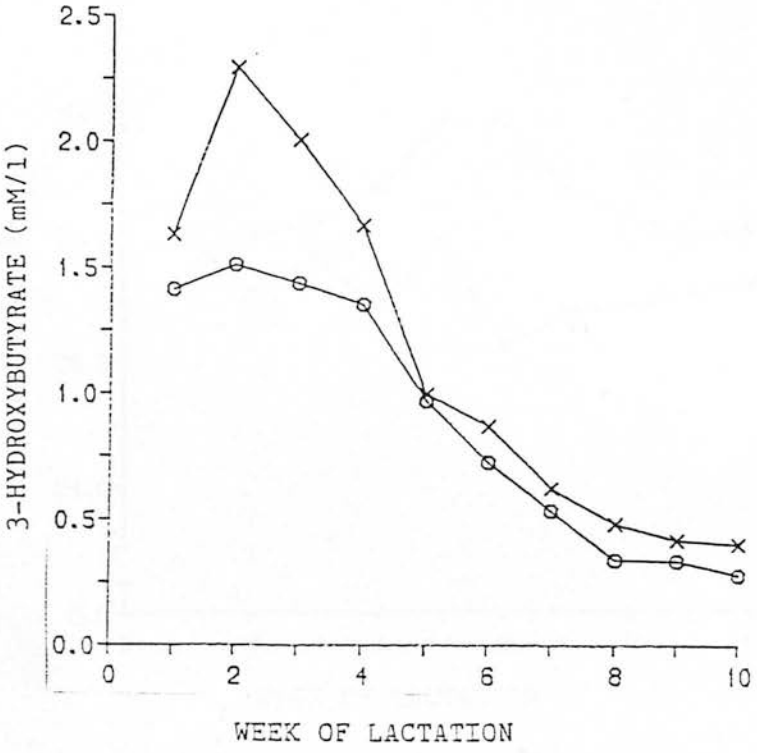


Figure 31. Mean plasma urea concentrations (mM/1) during lactation in ewes fed either a low x——x or high o——o protein diet (s.e.d. weeks 2, 4 and 10 = 0.589, 0.835, 0.876)

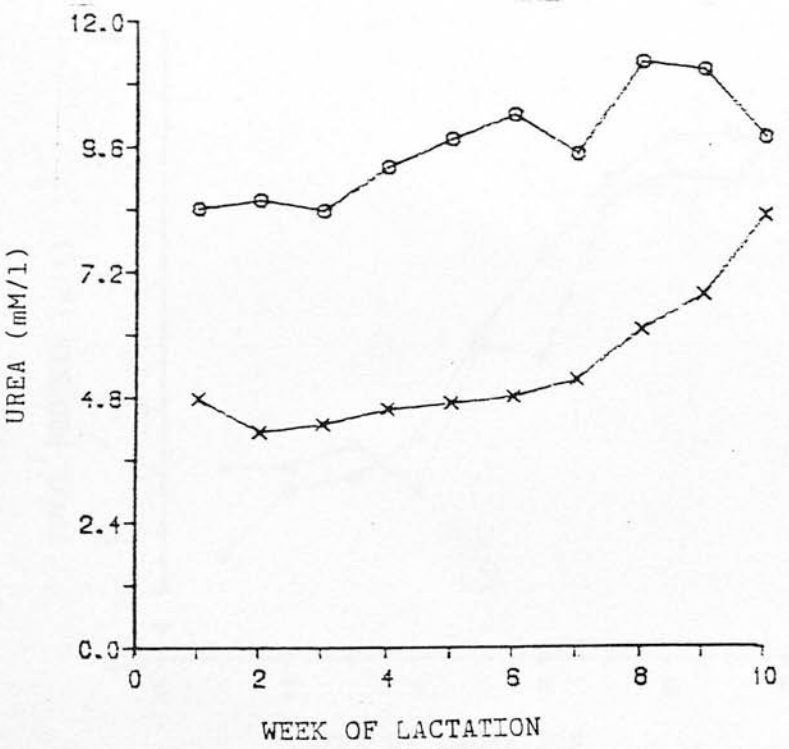


Figure 32. Mean plasma albumin concentrations (g/l) during lactation in ewes fed either a low x——x or high o——o protein diet (overall s.e.d. = 1.04)

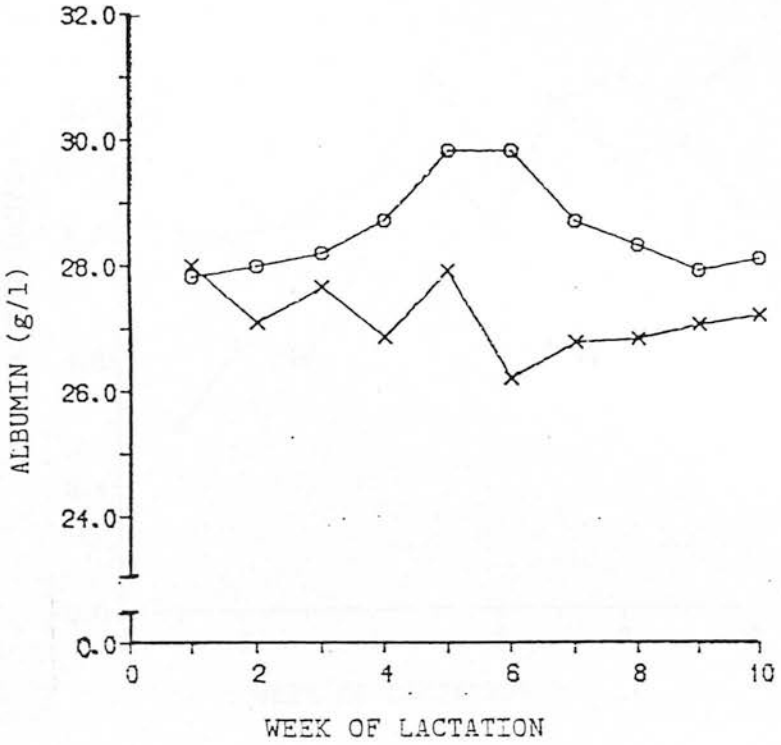


Figure 33. Mean plasma protein concentrations (g/l) during lactation in ewes fed either a low x——x or high o——o protein diet (overall s.e.d. = 2.96)

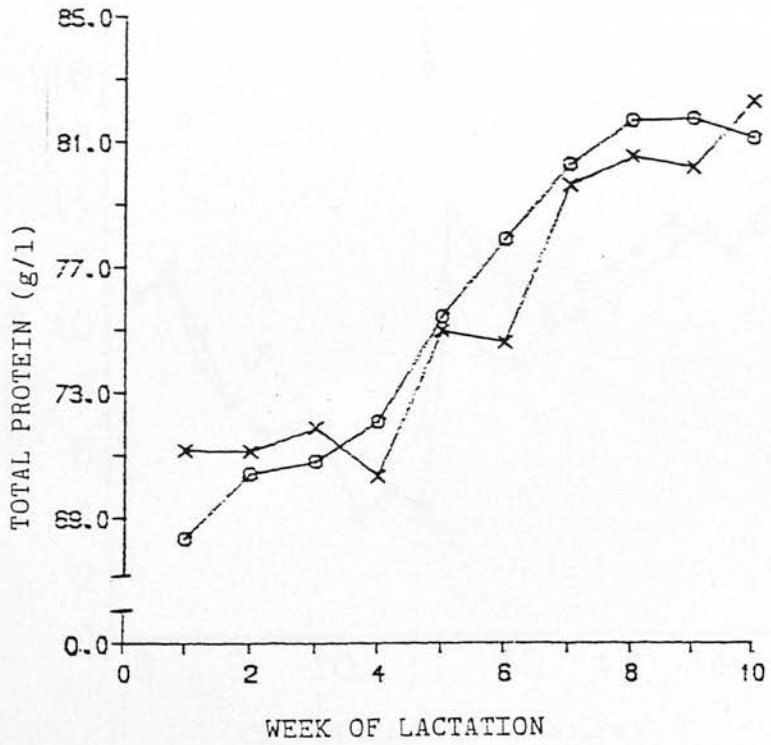


Figure 34. Mean plasma insulin concentrations (mU/l) during lactation in ewes fed either a low x——x or high o——o protein diet (s.e.d. weeks 2, 4 and 10 = 0.923, 1.169, 1.855)

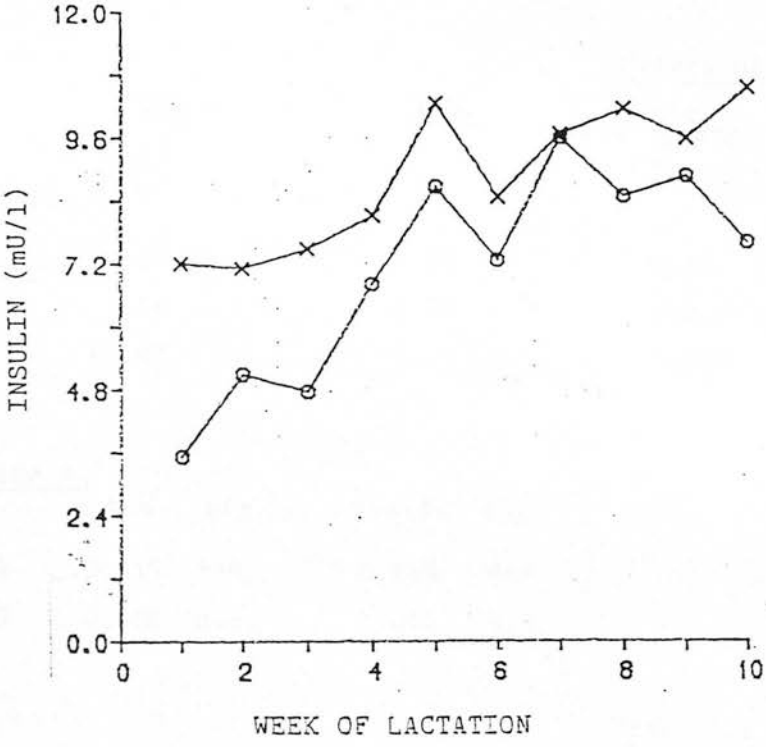


Figure 35. Changes in mean plasma insulin concentration (mU/l) during an 8 hour sampling period at week 2 of lactation in ewes fed either a low x——x or high o——o protein diet

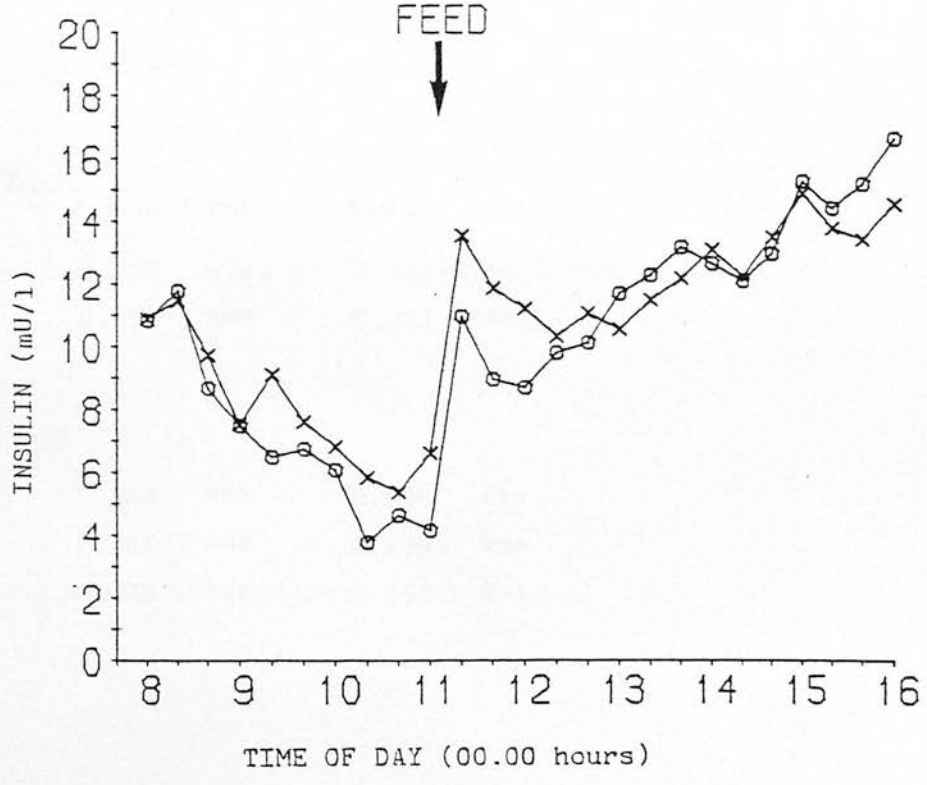


Table 9. Overall mean plasma insulin concentrations (mU/l) during the pre-feeding (samples 1-9) and post-feeding (samples 13-25) periods in L and H ewes during weeks 2, 4 and 10 of lactation.

PRE-FEEDING	LOW	HIGH	Effect of dietary protein content s.e.d.	sig.
Week of lactation				
2	8.26	7.38	0.966	n.s.
4	9.79	8.39	1.225	n.s.
10	10.87	8.17	1.508	n.s.

Effect of stage of lactation	s.e.d.	sig.	s.e.d.	sig.
Week 2 v. 4	0.319	***	0.259	***
Week 4 v. 10	0.380	n.s.	0.444	n.s.

POST-FEEDING	LOW	HIGH	Effect of dietary protein content s.e.d.	sig.
Week of lactation				
2	12.51	12.71	1.595	n.s.
4	13.03	13.52	1.544	n.s.
10	18.20	14.42	1.320	*

Effect of stage of lactation	s.e.d.	sig.	s.e.d.	sig.
Week 2 v. 4	0.330	n.s.	0.282	**
Week 4 v. 10	0.359	***	0.323	***

Effect of feeding				
Week 2	0.443	***	0.466	***
Week 4	0.361	***	0.479	***
Week 10	0.378	***	0.363	***

There was no significant difference with protein content of the diet in overall mean concentration. In L ewes, values were consistently higher than in H ewes although at the individual weeks tested the difference was significant only during week 2 ($P < 0.05$) of lactation.

Frequently collected samples:

At all 3 stages of lactation mean insulin concentration decreased prior to feeding, increased markedly immediately following feeding and then generally increased steadily over the remainder of the sampling period (shown at week 2 of lactation in Figure 35).

In general there was no difference with protein content of the diet in overall mean concentration (Table 9) during either the pre-feeding (samples 1-9) or post-feeding period (samples 13-25), although the value was significantly higher ($P < 0.05$) in L ewes than in H ewes during the post-feeding period in week 10 of lactation.

There was a progressive and generally significant increase in concentration associated with stage of lactation, during both the pre- and post-feeding periods in each diet group.

At all 3 stages of lactation values were markedly higher following feeding in each diet group ($P < 0.001$), the difference between pre- and post-feeding values being greatest during late lactation (week 10).

Growth Hormone

Weekly pooled samples:

Mean plasma GH concentration fluctuated throughout lactation in each diet group (Figure 36). There was no difference with protein content of the diet in overall mean concentration.

Frequently collected samples:

Plasma GH concentration fluctuated markedly throughout the day although levels tended to increase in response to feeding during early lactation (weeks 2 and 4) in each diet group (shown in week 2 of lactation in Figure 37).

Figure 36. Back-transformed mean plasma GH concentrations (ug/l) during lactation in ewes fed either a low x——x or high o——o protein diet (overall s.e.d. (expressed in log (value + 1) units) = 0.168)

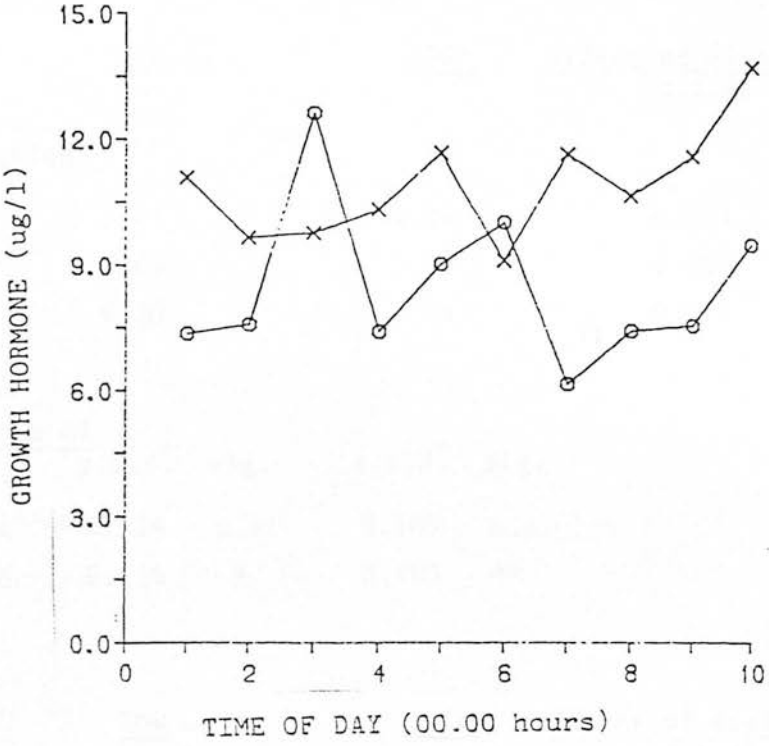


Figure 37. Changes in mean plasma GH concentration (ug/l) during an 8 hour sampling period at week 2 of lactation in ewes fed either a low x——x or high o——o protein diet

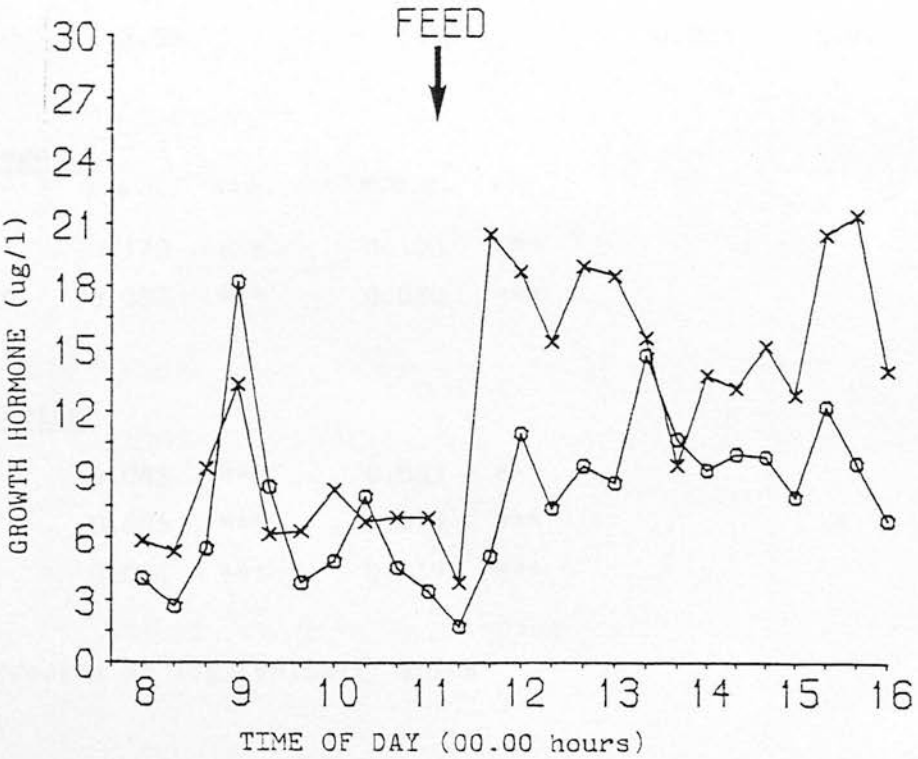


Table 10. Back-transformed overall mean plasma GH concentrations (ug/l) during the pre-feeding (samples 1-9) and post-feeding (samples 13-25) periods in L and H ewes during weeks 2, 4 and 10 of lactation.

PRE-FEEDING	LOW	HIGH	Effect of dietary protein content s.e.d.+	sig.
Week of lactation				
2	5.41	4.01	0.253	n.s.
4	4.64	3.99	0.201	n.s.
10	6.20	2.67	0.224	**

Effect of stage of lactation	s.e.d.+	sig.	s.e.d.+	sig.
Week 2 v. 4	0.114	n.s.	0.109	n.s.
Week 4 v. 10	0.116	*	0.103	**

POST-FEEDING	LOW	HIGH	Effect of dietary protein content s.e.d.+	sig.
Week of lactation				
2	10.38	5.97	0.415	n.s.
4	9.92	7.69	0.148	n.s.
10	2.95	1.50	0.233	n.s.

Effect of stage of lactation	s.e.d.+	sig.	s.e.d.+	sig.
Week 2 v. 4	0.120	n.s.	0.103	*
Week 4 v. 10	0.087	***	0.080	***
Effect of feeding				
Week 2	0.085	***	0.083	***
Week 4	0.086	***	0.073	***
Week 10	0.081	***	0.059	***

+s.e.d. expressed in log (value+1) units

Overall mean concentration was consistently higher in L ewes compared with H ewes during both the pre- and post-feeding periods, the difference being significant ($P < 0.01$) in the pre-feeding period during late lactation (week 10) (Table 10).

Changes in GH concentration with stage of lactation were inconsistent, although there was a marked decline in post-feeding values by late lactation (week 10).

There was a significant postprandial increase ($P < 0.001$) in overall mean concentration during early lactation (weeks 2 and 4). Conversely values decreased significantly ($P < 0.001$) following feeding during week 10 of lactation in each diet group.

Cortisol

Weekly pooled samples:

Mean plasma cortisol concentration remained almost constant throughout lactation (Figure 38). Protein content of the diet had no significant effect on overall mean concentration.

Frequently collected samples:

Mean cortisol concentration fluctuated throughout the day. At week 2 of lactation, levels increased prior to feeding and decreased following feeding but there was no consistent change during weeks 4 or 10 of lactation (shown at week 2 of lactation in Figure 39).

There was no difference with protein content of the diet in overall mean cortisol concentration either pre- or post-feeding, at any stage of lactation (Table 11). Levels were, however, generally higher in L ewes compared with H ewes.

Changes in overall concentration associated with stage of lactation were not marked, although values during late lactation (week 10) were lower compared with early lactation (week 2), particularly during the pre-feeding period.

Figure 38. Back-transformed mean plasma cortisol concentrations (ug/l) during lactation in ewes fed either a low x——x or high o——o protein diet (overall s.e.d. (expressed in log (value + 1) units) = 0.095)

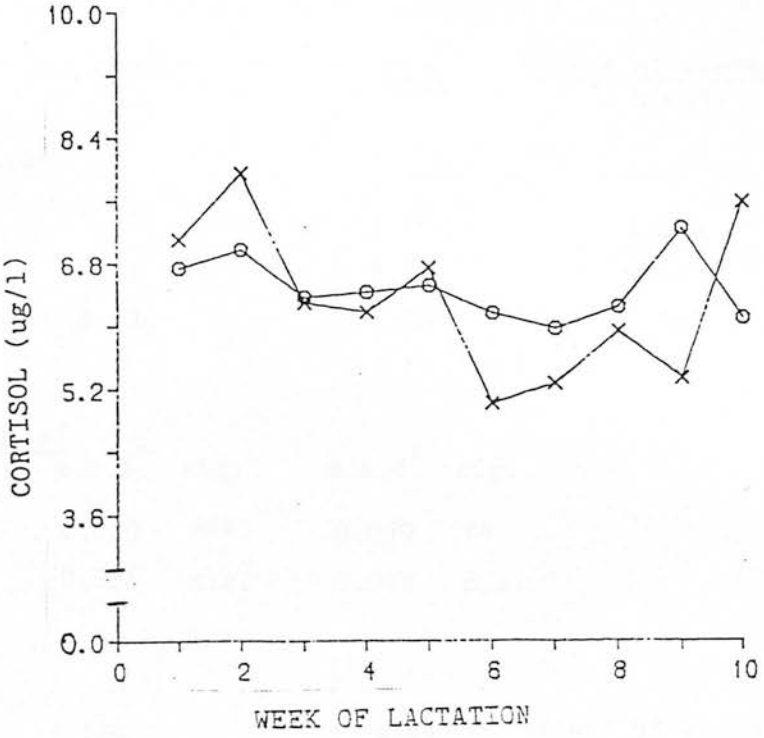


Figure 39. Changes in mean plasma cortisol concentration (ug/l) during an 8 hour sampling period at week 2 of lactation in ewes fed either a low x——x or high o——o protein diet

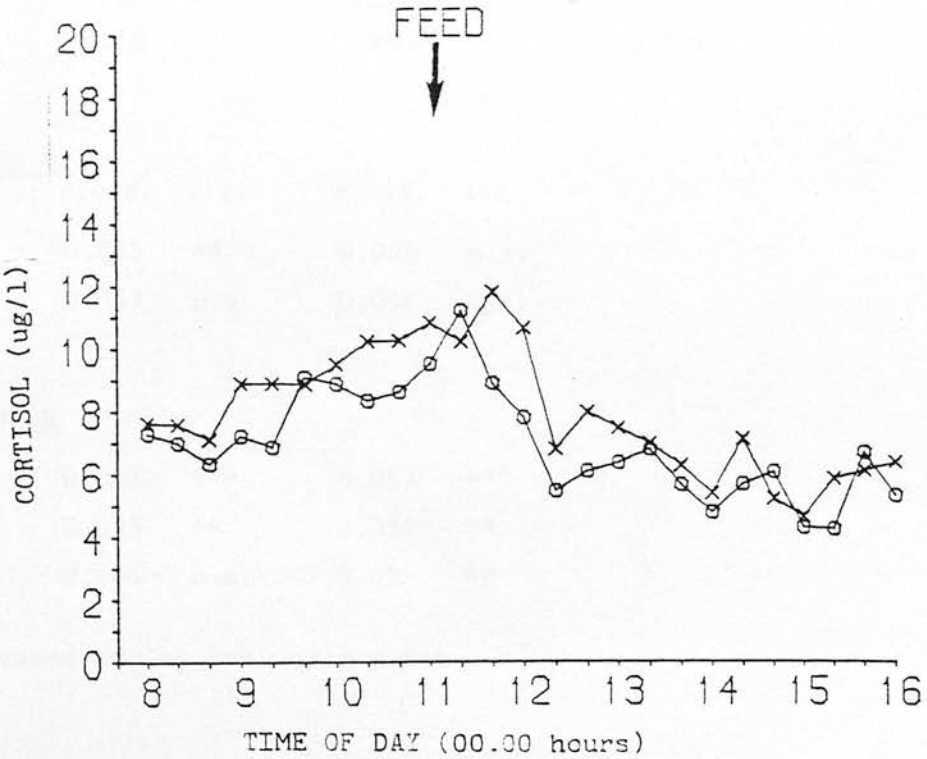


Table 11. Back-transformed overall mean plasma cortisol concentrations (ug/l) during the pre-feeding (samples 1-9) and post-feeding (samples 13-25) periods in L and H ewes during weeks 2, 4 and 10 of lactation.

PRE-FEEDING	LOW	HIGH	Effect of dietary protein content s.e.d.+	sig.
Week of lactation				
2	8.30	7.18	0.084	n.s.
4	6.21	5.89	0.107	n.s.
10	5.63	5.85	0.105	n.s.

Effect of stage of lactation	s.e.d.+	sig.	s.e.d.+	sig.
Week 2 v. 4	0.050	***	0.059	**
Week 4 v. 10	0.066	n.s.	0.046	n.s.

POST-FEEDING	LOW	HIGH	Effect of dietary protein content s.e.d.+	sig.
Week of lactation				
2	5.95	5.22	0.131	n.s.
4	5.15	4.95	0.131	n.s.
10	5.18	4.98	0.109	n.s.

Effect of stage of lactation	s.e.d.+	sig.	s.e.d.+	sig.
Week 2 v. 4	0.045	**	0.045	n.s.
Week 4 v. 10	0.067	n.s.	0.056	n.s.

Effect of feeding				
Week 2	0.051	***	0.053	***
Week 4	0.055	**	0.054	**
Week 10	0.064	n.s.	0.051	**

+s.e.d. expressed in log (value+1) units

There was a generally significant postprandial decrease in overall concentration, the difference being largest during early lactation (week 2) in each of the diet groups.

Prolactin

Weekly pooled samples:

Mean plasma prolactin concentration decreased throughout lactation in each diet group (Figure 40).

There was no significant difference with protein content of the diet in overall mean concentration. In L ewes values were consistently higher compared with H ewes, although in the tested weeks differences were not significant.

Frequently collected samples:

Plasma prolactin concentration generally decreased prior to feeding, increased markedly around the time of feeding and increased gradually over the remainder of the sampling period at all 3 stages of lactation (shown at week 2 of lactation in Figure 41), although no increase around the time of feeding was observed during late lactation (week 10).

Protein content of the diet had no significant effect on overall mean prolactin concentration either before or after feeding, although values were generally higher in L ewes than H ewes, the difference being greatest during early lactation (week 2) (Table 12).

In general there was a progressive and significant decrease in overall mean prolactin levels associated with stage of lactation.

There was a postprandial decrease in concentration which was significant in week 2 of lactation ($P < 0.05$) in each diet group, although not on any other occasion with the exception of L ewes during week 10 of lactation.

Figure 40. Back-transformed mean plasma prolactin concentrations (ug/l) during first 10 weeks of lactation in ewes fed a low x——x or high o——o protein diet (s.e.d. (expressed in log units) weeks 2, 4 and 10 = 0.40, 0.35, 0.10)

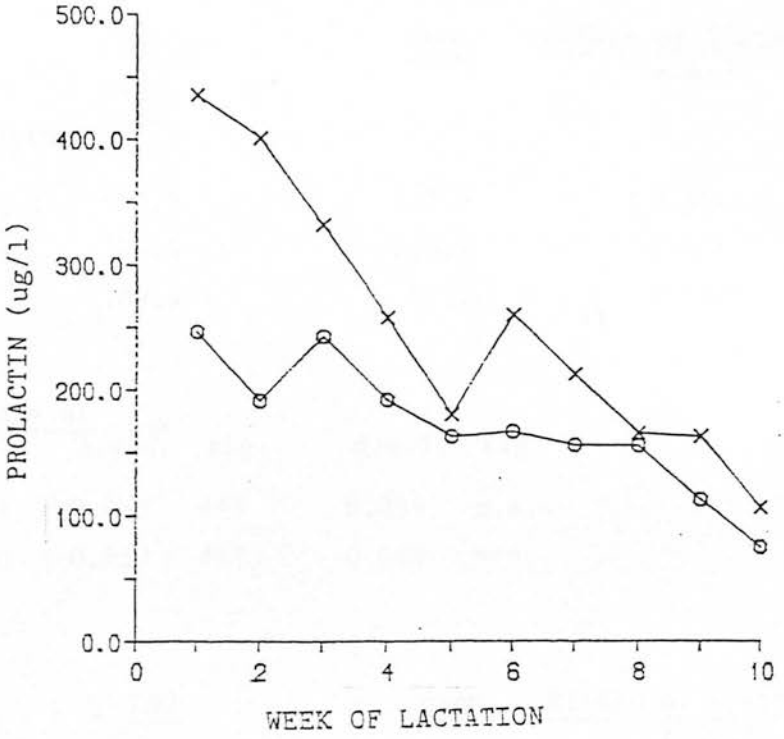


Figure 41. Changes in mean plasma prolactin concentration (ug/l) during an 8 hour sampling period at week 2 of lactation in ewes fed either a low x——x or high o——o protein diet

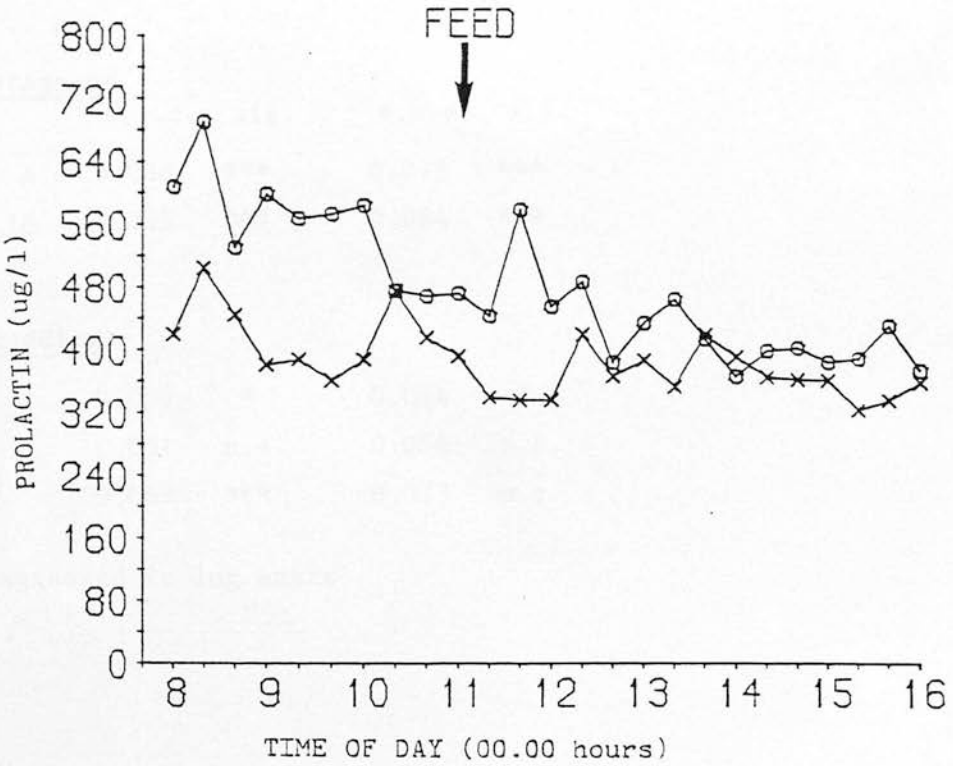


Table 12. Back-transformed overall mean plasma prolactin concentrations (ug/l) during the pre-feeding (samples 1-9) and post-feeding (samples 13-25) periods in L and H ewes during weeks 2, 4 and 10 of lactation.

PRE-FEEDING	LOW	HIGH	Effect of dietary protein content s.e.d.+	sig.
Week of lactation				
2	403.4	221.9	0.351	n.s.
4	325.4	223.2	0.291	n.s.
10	109.4	96.7	0.129	n.s.

Effect of stage of lactation	s.e.d.+	sig.	s.e.d.+	sig.
Week 2 v. 4	0.033	***	0.059	n.s.
Week 4 v. 10	0.059	***	0.068	***

POST-FEEDING	LOW	HIGH	Effect of dietary protein content s.e.d.+	sig.
Week of lactation				
2	353.2	184.7	0.331	n.s.
4	307.4	248.9	0.197	n.s.
10	69.9	88.1	0.153	n.s.

Effect of stage of lactation	s.e.d.+	sig.	s.e.d.+	sig.
Week 2 v. 4	0.038	***	0.075	***
Week 4 v. 10	0.065	***	0.064	***

Effect of feeding				
Week 2	0.059	*	0.064	*
Week 4	0.051	n.s.	0.066	n.s.
Week 10	0.068	***	0.073	n.s.

+s.e.d. expressed in log units

Tri-iodothyronine

Weekly pooled samples:

Plasma T^3 concentration remained fairly constant throughout lactation in each diet group (Figure 42). There was no significant difference with protein content of the diet in overall mean concentration. In L ewes, values were consistently lower than in H ewes.

Thyroxine

Weekly pooled samples:

Plasma T^4 concentration increased during early lactation in each diet group. Values declined slightly during mid- and late lactation in L ewes (Figure 43) while in H ewes values continued to increase during mid-lactation before decreasing after week 7 of lactation (Figure 43).

Protein content of the diet had no significant effect on overall mean concentration. In L ewes, values were consistently lower than in H ewes between weeks 4 and 10 of lactation.

Figure 42. Mean plasma T^3 concentrations (ug/l) during lactation in ewes fed either a low x——x or high o——o protein diet (overall s.e.d. = 0.072)

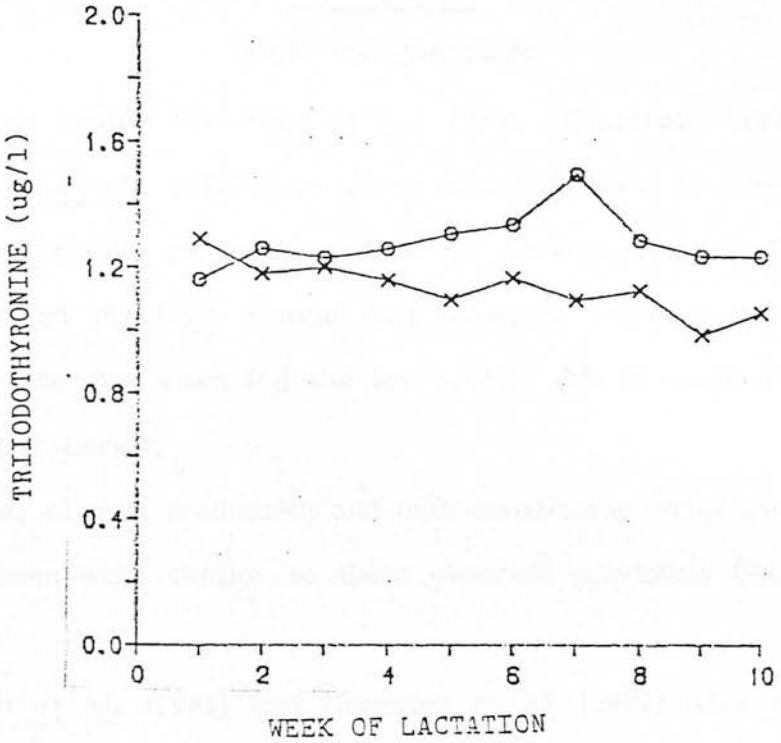
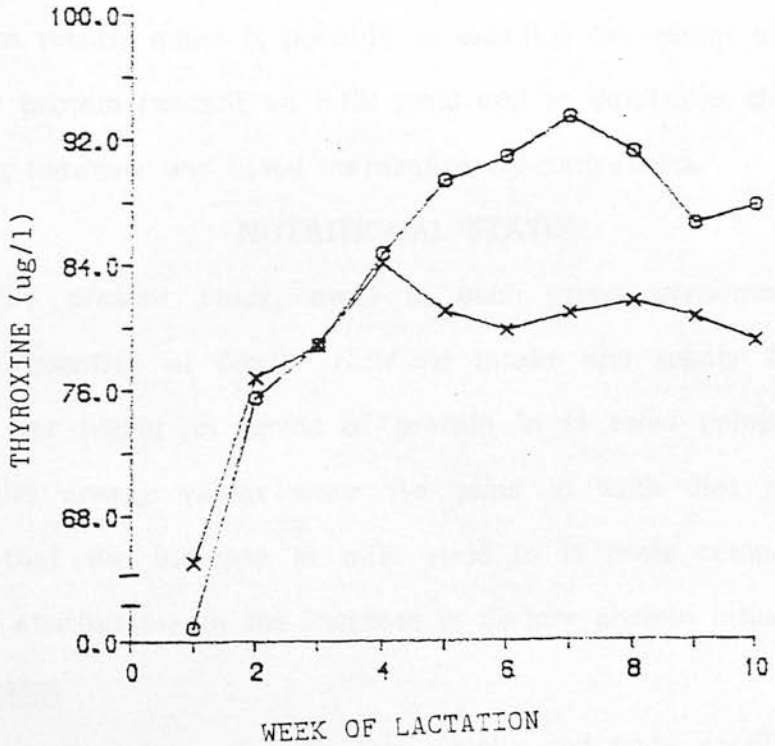


Figure 43. Mean plasma T^4 concentrations (ug/l) during lactation in ewes fed either a low x——x or high o——o protein diet (s.e.d. weeks 2, 4 and 10 = 6.49, 7.57, 5.51)



DISCUSSION

MILK PRODUCTION

Previous results (Robinson et al., 1974; Calderton Cortes et al., 1977; Cowan et al., 1981) have shown that milk yield is increased when ewes are fed a high compared with a low protein diet. In the present study ewes fed the high protein diet (H ewes) produced proportionally 0.14 more milk than ewes fed the low protein diet (L ewes) over the 10 week lactation period.

Profiles of milk production and milk constituent composition in both L and H ewes were similar to those observed previously (Peart et al., 1972).

Cowan et al. (1981) and Gonzalez et al. (1982) have shown that when milk production is stimulated by increasing dietary protein level, milk protein content is also higher, while milk fat and lactose contents are similar. The results of the present study are consistent with these observations.

These results make it possible to examine the effect of differences in dietary protein content on milk yield and to determine the effects on circulating hormone and blood metabolite concentrations.

NUTRITIONAL STATUS

In the present study, ewes in each group consumed the same restricted quantity of feed. Nutrient intake and supply to the small intestine was higher in terms of protein in H ewes compared with L ewes, while energy values were the same in each diet group. This suggests that the increase in milk yield in H ewes compared with L ewes was attributable to the increase in dietary protein intake.

Energy status

The marked loss of both live weight and body condition in each diet group during the first four weeks of the lactation period indicates

that ewes in both diet groups were mobilising a substantial amount of body tissue in order to supply nutrients for milk production. This result is consistent with the elevated NEFA and 3-OHB levels during early lactation which indicate that the rate of adipose tissue mobilisation was highest during this period. The absence of any significant difference in live weight or body condition losses (relatively crude measures) between L and H ewes during early lactation implies that nutrient supply from mobilisation of body tissue was similar in both diet groups. However, the substantially higher NEFA and, to a lesser extent, 3-OHB concentrations, in L ewes compared with H ewes during this period suggest that the rate of adipose tissue mobilisation was in fact higher in L than in H ewes.

These results suggest that the increase in milk yield in H ewes compared with L ewes is not a function of an increase in the supply of nutrients from adipose tissue mobilisation and in fact rate of adipose tissue utilisation may have been lower in the higher yielding group (i.e. H ewes).

Previous workers have demonstrated several effects of increased protein levels on nutrient metabolism. Robinson et al. (1974; 1978) showed that stimulation of milk yield by increasing dietary protein content was associated with an increase in body tissue utilisation. More recent work by Cowan et al. (1981), however, did not show any difference in amount of adipose tissue utilisation between groups of ewes fed different amounts of dietary protein. It was postulated that stimulation of milk yield by increasing dietary protein level may be attributable to an increase in the efficiency of utilisation of energy for milk production released by tissue mobilisation. Other effects of increasing dietary protein level have also been observed e.g. effects on food intake and digestibility (Oldham, 1984).

The inter-relationship between energy and protein is complicated and with the information available it is impossible to draw any firm conclusions concerning the mode of action of increased dietary protein on increased milk yield in the present study. Nevertheless, it seems likely that there was an increase in the efficiency of use of nutrients in the higher yielding group, but measurements, of absorbed nutrient supply are required to confirm or disprove this.

Protein status

It is unlikely that differences in plasma urea concentration were related to differences in tissue protein mobilisation between diet groups throughout lactation as dietary protein levels were almost certainly not limiting milk production over the majority of the lactation period, except perhaps around the peak of lactation in each group. There were no differences with dietary protein content in either of the indices of long-term protein status (albumin and total protein) at any stage of lactation. Therefore the markedly higher circulating urea levels in H ewes than in L ewes suggests that the degree of excess of amino acid supply in relation to requirements was greater in H ewes than L ewes. Furthermore, the increase in urea and total protein concentrations throughout lactation suggests that the amount of amino acids surplus to requirements was increasing as lactation progressed; this would be expected in view of the decrease in milk production and milk protein production after week 4 of lactation.

It is perhaps noteworthy that while plasma protein content increased during lactation, plasma albumin concentration remained fairly constant. As the total protein component of plasma contains mainly albumin and globulin, this suggests that plasma globulin levels were increasing during lactation; the biological significance of this result is not clear.

In summary, there was clear evidence that ewes in both diet groups were utilising adipose tissue during early lactation. However, stimulation of milk yield by increasing dietary protein content was not apparently associated with an increase in the rate of adipose tissue utilisation, but may have been associated with an increase in the efficiency of utilisation of substrates mobilised from body tissue. It is possible that examination of hormone levels will help to explain how the difference in milk production is controlled.

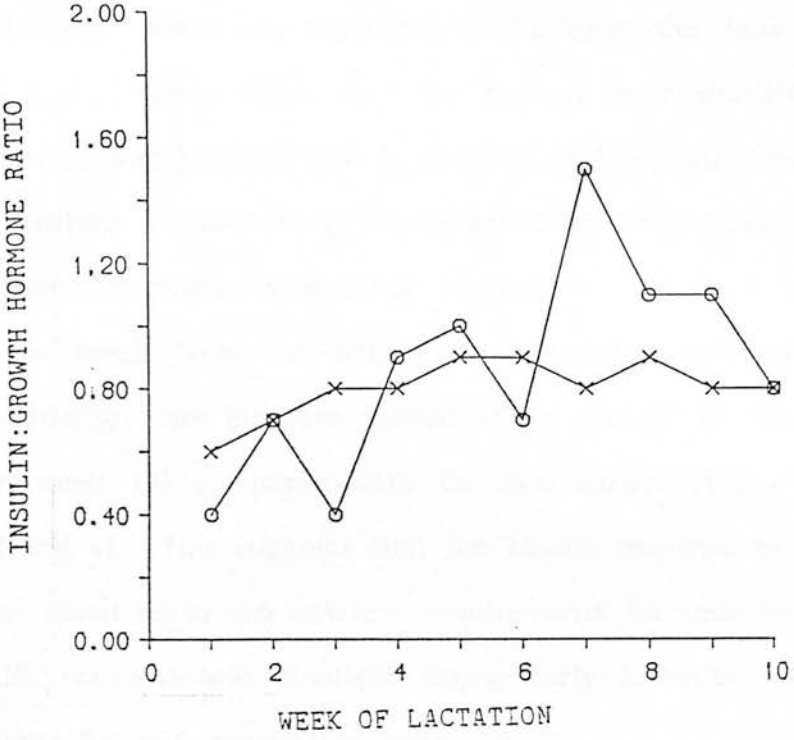
HORMONE STATUS

The fact that plasma insulin levels were lower in H ewes than in L ewes during early lactation is perhaps surprising in view of the fact that the NEFA and 3-OHB results indicated that rate of adipose tissue mobilisation was greater in L ewes compared with H ewes. Examination of GH levels does not help to improve understanding as levels were very variable during early lactation, although there was a tendency for GH levels to be lower in H ewes than in L ewes, which is consistent with trends observed in NEFA and 3-OHB levels.

The absence of any marked difference in the relationship between insulin and GH (Figure 43a) between diet groups is consistent with the view that the increase in milk yield is not related to a large increase in amount of nutrients supplied from mobilisation of adipose tissue.

The absence of any consistent change in GH levels during lactation despite the marked change in rate of tissue mobilisation (as estimated by NEFA and 3-OHB levels) around week 4 of lactation is consistent with the results observed in the litter size comparison. As previously suggested this effect may be related to changes in receptor number and/or target tissue sensitivity, factors which require further investigation.

Figure 43a. Mean plasma insulin : geometric GH ratio during lactation in ewes fed either a low x——x or high o——o protein diet.



The overall increase in plasma insulin levels in each diet group during lactation would be expected to increase the rate of nutrient accretion and it seems likely that the increase in insulin:GH ratio (as a result of increasing insulin levels in relation to fairly constant GH levels) may be involved in controlling the switch from body tissue mobilisation to body tissue accretion as lactation proceeds.

Insulin levels were consistently and markedly increased following feeding, although this increase tended to be greater at the end of the lactation (week 10) compared with the two earlier stages of lactation (weeks 2 and 4). This suggests that the insulin response to feeding may have been sensitive to the nutrient requirements for milk production i.e. the insulin response was smallest during early lactation when nutrient requirements for milk production were highest.

The increase in GH levels which occurred after feeding during weeks 2 and 4 of lactation is interesting as previous studies have shown a decrease in GH levels during the post prandial period (Trenkle, 1978). However, there was a tendency for GH levels to increase following feeding in the litter size study, particularly in T ewes (i.e. the higher-yielding ewes). Work has shown that GH may be stimulated by infusion of several blood metabolites including amino acids (Trenkle, 1978). Post prandial increases in circulating levels of blood metabolites could, in part, explain the increase in GH levels after feeding in the present study, although the absence of any post prandial increase in GH levels in each diet group during week 10 of lactation is not entirely consistent with this suggestion. One possible explanation is that there may be a change in sensitivity to amino acid or other blood metabolite stimulus with change of stage of lactation. Whatever the reason for the increased GH levels following feeding during early lactation the net result was an increase in GH at a time when nutrient requirements for

milk production were highest and direction of nutrients towards milk production at the expense of body tissue was most necessary. The increase in GH level after feeding, at this stage of lactation, may balance the anabolic effects of increased insulin concentration required on nutrient metabolism to ensure utilisation of ingested nutrients. Previous work has demonstrated that GH antagonises the effects of insulin (Vernon, 1982). However the mechanism whereby GH is stimulated and the precise mode of action of this hormone is not clear.

These effects can be summarised as follows: the higher milk production stimulated by increasing the dietary protein content was not associated with any marked difference in overall plasma insulin or GH levels, which is consistent with absence of significant differences between the two diet groups in terms of rate of body tissue utilisation.

While differences in milk production are not explicable in terms of insulin and GH, the switch in direction of nutrients towards milk or body tissue may be related to the change in relationship between insulin and GH levels during lactation. The relationship between insulin and GH during the post prandial period may be important in relation to nutrient partitioning.

Despite the fact that cortisol is known to be involved in the control of energy and protein metabolism (Trenkle, 1981) the higher milk production in H compared with L ewes was not associated with any difference in overall mean weekly cortisol concentration. The marginally higher cortisol levels at the start of lactation in both diet groups may reflect a requirement for an increased rate of gluconeogenesis in response to increased glucose requirements for lactose production during early lactation. The consistently higher circulating cortisol concentrations before feeding compared with post-feeding on the other hand suggests that cortisol may be involved in stimulating supplies of

nutrients during short-term periods of nutrient deficiency i.e. prior to feeding on a once/day feeding regime.

With regard to protein metabolism, the increase in insulin concentration during lactation in relation to the slight decline in cortisol concentration would be expected to exert an increasingly anabolic influence on circulating amino acids, which is consistent with the increases in the indices of protein status (Sykes, 1978) and decreasing milk and milk protein production with advancing stage of lactation.

The relationship between GH and cortisol may be important with regard to the availability of both energy and protein yielding substrates for milk production. There was very little change in concentration of either hormone prior to feeding, although there was a marked increase in the GH:cortisol ratio immediately following feeding during early lactation. In addition to the postulated role of GH on energy metabolism it is possible that GH may also be stimulating amino acid uptake at the mammary gland during this period (Bauman and McCrutcheon, 1986). The fact that cortisol levels are not stimulated during this period again suggests that this hormone is not directly involved in changes in nutrient metabolism postprandially.

As in the litter size comparison, reported earlier, the pattern of prolactin levels generally reflected the pattern of milk production. There is no obvious explanation for the generally lower although non-significant prolactin values in H than in L ewes.

A similar trend in circulating prolactin levels was observed during the 8 hour sampling period at each stage of lactation, again, as seen in the litter comparisons. This may be due to a daily rhythm of prolactin release unrelated to feeding. There is no evidence from this experiment or the literature to suggest that prolactin is involved in any short-term regulation of nutrient metabolism in ruminant species.

Finally the difference in milk production between H and L ewes was not associated with any significant difference in overall weekly T3 or T4 levels. The marked increase in T4 levels during early lactation in both diet groups with advancing stage of lactation is consistent with previous results (Hart et al., 1978) and suggests that reduced T4 levels during early lactation may have a permissive role in milk production. However, this contrasts with the higher levels of thyroid hormones in H ewes than in L ewes during the latter half of lactation. While not statistically significant the difference in thyroid hormone levels during this period may have some influence on rates of metabolism of tissues or on the action of other hormones. Further information is clearly required concerning the mode of action of thyroid hormones and associated receptors during lactation.

CONCLUSION

Stimulation of milk production by increasing the dietary protein content was not apparently associated with any parallel increase in mobilisation of substrates from body tissues; indeed the reverse may have occurred. Hormone levels were generally similar in the two diet groups during lactation. Changes in hormone concentrations during lactation again highlight the hormonal inter-relationships that may be most significant in the control of nutrient availability and milk yield i.e. GH/insulin. Furthermore, the role of GH following feeding may be important in relation to the partition of nutrients during the postprandial period when nutrient requirements are high as they are during early lactation.

CHAPTER 6

THE EFFECT OF EWE GENOTYPE (EXPT 4)

RESULTS

Data collected during week 9 of lactation has been excluded from all statistical analysis, owing to a temporary change of diet which reduced feed intakes.

MILK PRODUCTION

The effect of number of lambs born in relation to milk yield during week 1 was examined in East Friesland (EF) ewes. There was no difference in daily milk yield in EF ewes which bore triplets and suckled twins (mean=2.38 kg/day; n = 3; s.e. = 0.297) compared with EF ewes which bore and suckled twin lambs (mean=2.30 kg/day; n=5; s.e. = 0.209). No equivalent comparison was necessary in Scottish Blackface (SBF) ewes as all ewes bore twin lambs.

Mean daily milk yield increased during early lactation, remained elevated between weeks 2 and 8 and declined steadily until the end of the 14 week lactation period in each ewe breed (Figure 44). Peak yield was attained during week 5 of lactation in SBF ewes and during week 6 of lactation in EF ewes.

There was no difference in overall mean (i.e. grand mean over the whole 14 week lactation) daily milk production in EF ewes compared with SBF ewes (2.48 v. 2.37 kg/day; s.e.d. = 0.155; $P > 0.05$). During the last 4 weeks of the lactation study values were consistently higher in EF ewes than in SBF ewes, the difference being significant during week 14 ($P < 0.05$) of lactation (weeks 2, 6 and 14 being those selected for the individual statistical comparisons in this study).

Figure 44. Mean daily milk production (kg/day) during lactation in EF x——x and SBF ewes o——o (s.e.d. weeks 2, 6 and 14 = 0.194, 0.268, 0.177)

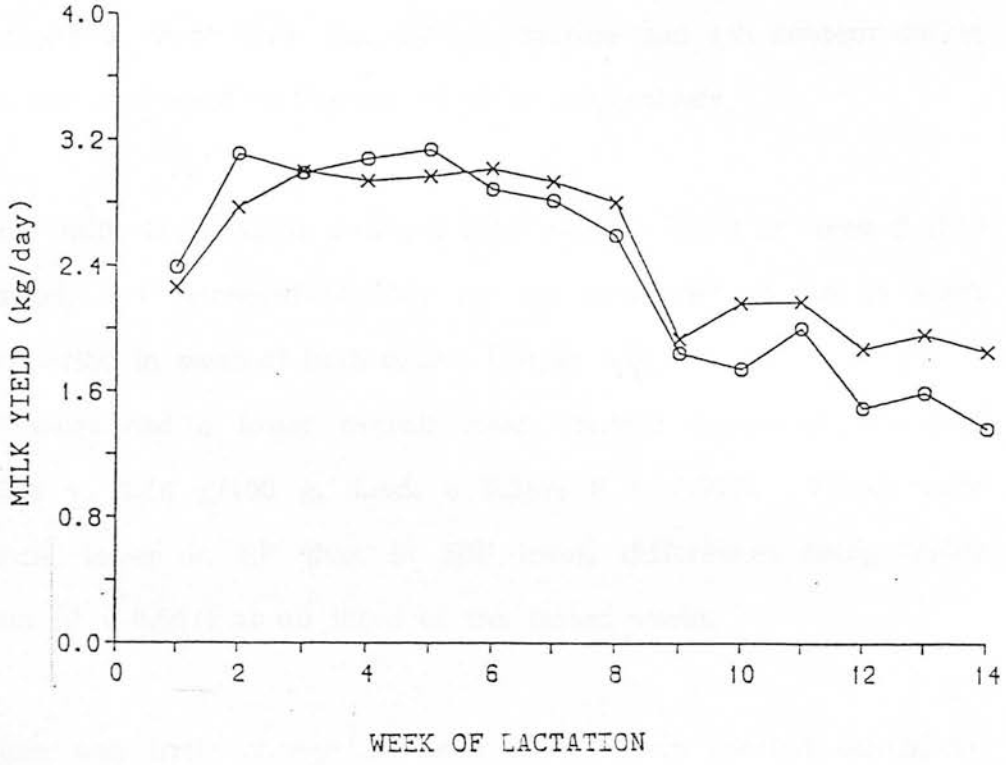
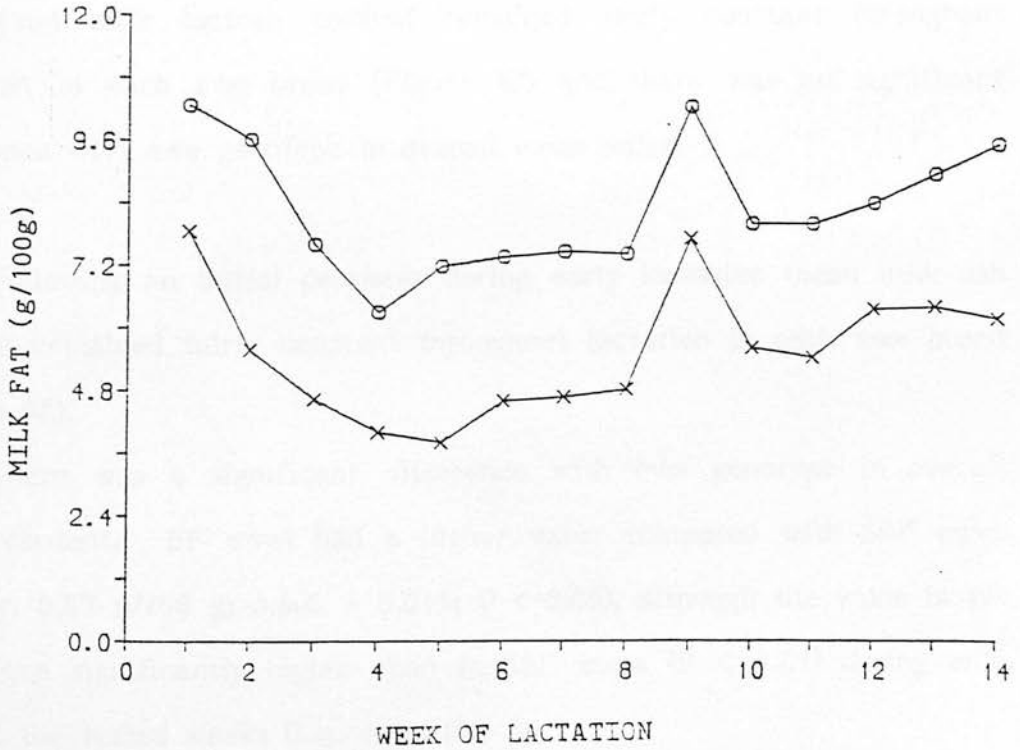


Figure 45. Mean milk fat contents (g/100 g) during lactation in EF x——x and SBF ewes o——o (s.e.d. weeks 2, 6 and 14 = 0.573, 0.586, 0.697)



MILK COMPOSITION

Changes in mean milk fat, protein, lactose and ash content during lactation are illustrated in Figures 45 to 48 respectively.

Fat

Mean milk fat content declined until week 4 (SBF) or week 5 (EF) of lactation, and increased steadily for the remainder of the 14 week lactation period in ewes of both breeds (Figure 45).

EF ewes had a lower overall mean content compared with SBF ewes (5.38 v. 8.16 g/100 g; s.e.d. = 0.369; $P < 0.001$). Values were consistently lower in EF than in SBF ewes, differences being highly significant ($P < 0.001$) at all three of the tested weeks.

Protein

There was little change in mean milk protein content throughout lactation in either ewe breed (Figure 46) and there was no significant difference with ewe genotype in overall mean value.

Lactose

Mean milk lactose content remained fairly constant throughout lactation in each ewe breed (Figure 47) and there was no significant difference with ewe genotype in overall mean value.

Ash

Following an initial decrease during early lactation mean milk ash content remained fairly constant throughout lactation in each ewe breed (Figure 48).

There was a significant difference with ewe genotype in overall mean content. EF ewes had a higher value compared with SBF ewes (0.87 v. 0.83 g/100 g; s.e.d. = 0.015; $P < 0.05$), although the value in EF ewes was significantly higher than in SBF ewes ($P < 0.01$) during only one of the tested weeks (i.e. week 6).

Figure 46. Mean milk protein contents (g/100 g) during lactation in EF x——x and SBF ewes o——o (overall s.e.d. = 0.179)

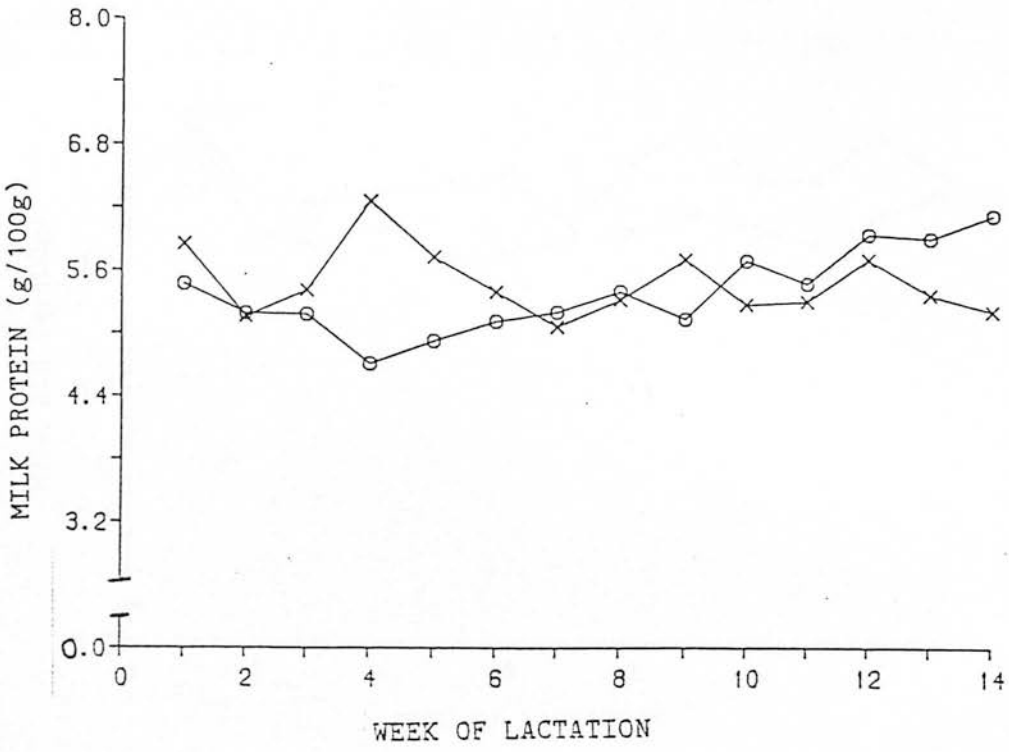


Figure 47. Mean milk lactose contents (g/100 g) during lactation in EF x——x and SBF ewes o——o (overall s.e.d. = 0.127)

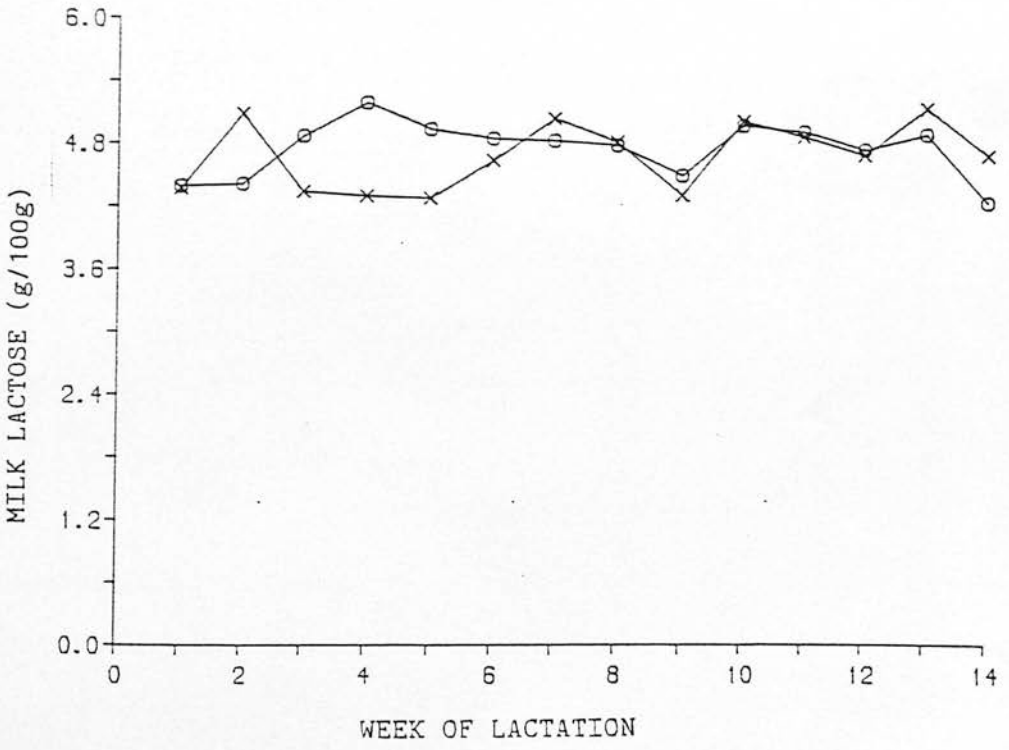
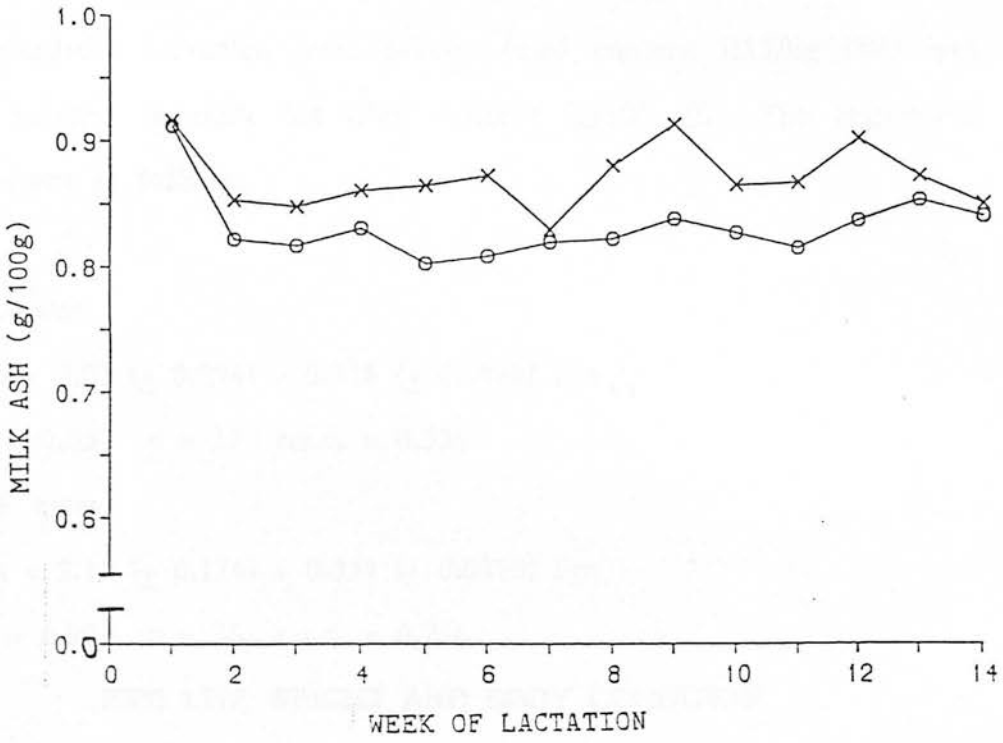


Figure 48. Mean milk ash contents (g/100 g) during lactation in EF x——x and SBF ewes o——o (s.e.d. weeks 2, 6 and 14 = 0.017, 0.022, 0.024)



MILK ENERGY CALCULATION

Throughout lactation milk energy (Em) content (MJ/kg DM) was linearly related to milk fat (Fm) content (g/100 g). The regression equations are as follows:

1. EF ewes

   $Em = 2.01 (\pm 0.294) + 0.318 (\pm 0.0484) Fm$

   $r^2 = 0.552$ n = 37 r.s.d. = 0.534

2. SBF ewes

   $Em = 2.15 (\pm 0.174) + 0.354 (\pm 0.0199) Fm$

   $r^2 = 0.903$ n = 36 r.s.d. = 0.266

EWE LIVE WEIGHT AND BODY CONDITION

Ewes of both breeds gained live weight throughout lactation, rate of gain being greater during the first 6 weeks compared with the last 8 weeks of the 14 week lactation study. There was no difference with ewe genotype in ewe live weight either postpartum or during week 6 or 14 of lactation (Table 13).

Both ewe breeds lost body condition during early lactation and gained condition during mid- and late lactation, rate of condition gain being slightly lower in EF than in SBF ewes. Body condition scores were consistently lower in EF ewes compared with SBF ewes, the difference being significant postpartum ($P < 0.05$) and during week 14 ($P < 0.001$) of lactation (Table 13).

LAMB LIVE WEIGHT

There was no difference with ewe genotype in mean lamb live weight at birth (Table 14). However, by week 14 of lactation EF lambs had a significantly higher live weight compared with SBF lambs (Table 14). Rate of live weight gain was significantly higher in EF than in SBF lambs during the last 8 weeks of the 14 week lactation study (Table 14).

Table 13. Mean ewe live weights (kg) and body condition scores postpartum, and at weeks 6 and 14 of lactation.

Ewe breed	EF	SBF	s.e.d.	sig.
Live weight (kg)				
Postpartum	55.8	54.4	2.44	n.s.
Week 6	59.8	60.1	3.04	n.s.
Week 14	61.7	60.5	2.90	n.s.
Body condition score				
Postpartum	1.75	2.00	0.099	*
Week 6	1.73	1.85	0.061	n.s.
Week 14	1.89	2.36	0.081	***

Table 14. Mean lamb live weights (kg), at birth and 14 weeks of age and mean lamb live weight gains (g/day) at 0-6 and 6-14 weeks of lactation

Lamb breed	EF	SBF	s.e.d.	sig.
Live weight (kg)				
Birth	3.7	3.7	0.21	n.s.
14 weeks	27.9	23.7	0.97	***
Live weight gain (g/day)				
0-6 weeks	238	244	11.0	n.s.
6-14 weeks	274	199	11.6	***

VOLUNTARY FOOD INTAKE

There was no difference with ewe genotype in overall mean ewe voluntary intake during the first 6 weeks of the lactation study (Table 15). Following week 6 of lactation feed intakes of ewes could not be measured accurately as lambs were beginning to consume their dams' feed, however total intakes were higher for EF ewes and lambs than for SBF ewes and lambs during the last 8 weeks of the 14 week study.

WEEKLY POOLED BLOOD METABOLITE CONCENTRATIONS

Glucose

Mean plasma glucose concentration tended to increase during early lactation and generally declined during the remainder of the lactation period in each ewe breed (Figure 49).

There was a significant difference with ewe genotype in overall mean concentration. EF ewes had a lower value compared with SBF ewes (2.73 v. 2.93 mM/l; s.e.d. = 0.060; $P < 0.01$). In the individual weeks tested values were lower in EF ewes than in SBF ewes only during week 2 ($P < 0.05$) of lactation.

Non-esterified fatty acids

There was a decrease in mean plasma NEFA concentration between week 1 and 8 of lactation and an increase between week 10 and 14 of lactation in each ewe breed (Figure 50). Ewe genotype had no significant effect on overall mean concentration.

3-hydroxybutyrate

Mean 3-OHB concentration declined sharply during early lactation and then generally decreased gradually for the remainer of the 14 week lactation period in each ewe breed (Figure 51).

EF ewes had a lower value compared with SBF ewes (0.43 v. 0.67 mM/l; s.e.d. (expressed in log (value+1) units) = 0.045; $P < 0.01$). However, there were no significant differences between breeds at any of

Table 15. Mean voluntary food intakes (kg/day) at 0-6 and 6-14 weeks of lactation

Ewe breed	EF	SBF	s.e.d.	sig.
Feed intake (kg/day)				
0-6 weeks	3.77	3.71	0.233	n.s.
6-14 weeks	4.94	4.58	0.116	**

Figure 49. Mean plasma glucose concentrations (mM/l) during lactation in EF x——x and SBF ewes o——o (s.e.d. weeks 2, 6 and 14 = 0.152, 0.197, 0.116)

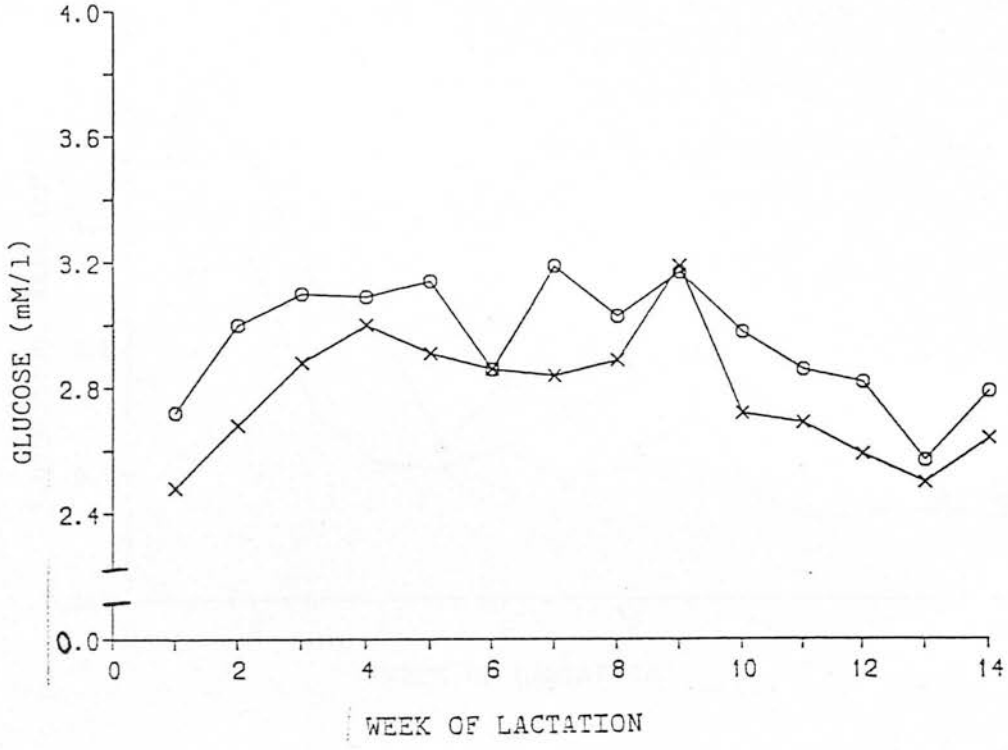


Figure 50. Mean plasma NEFA concentrations (uM/l) during lactation in EF x——x and SBF ewes o——o (overall s.e.d. = 59.4)

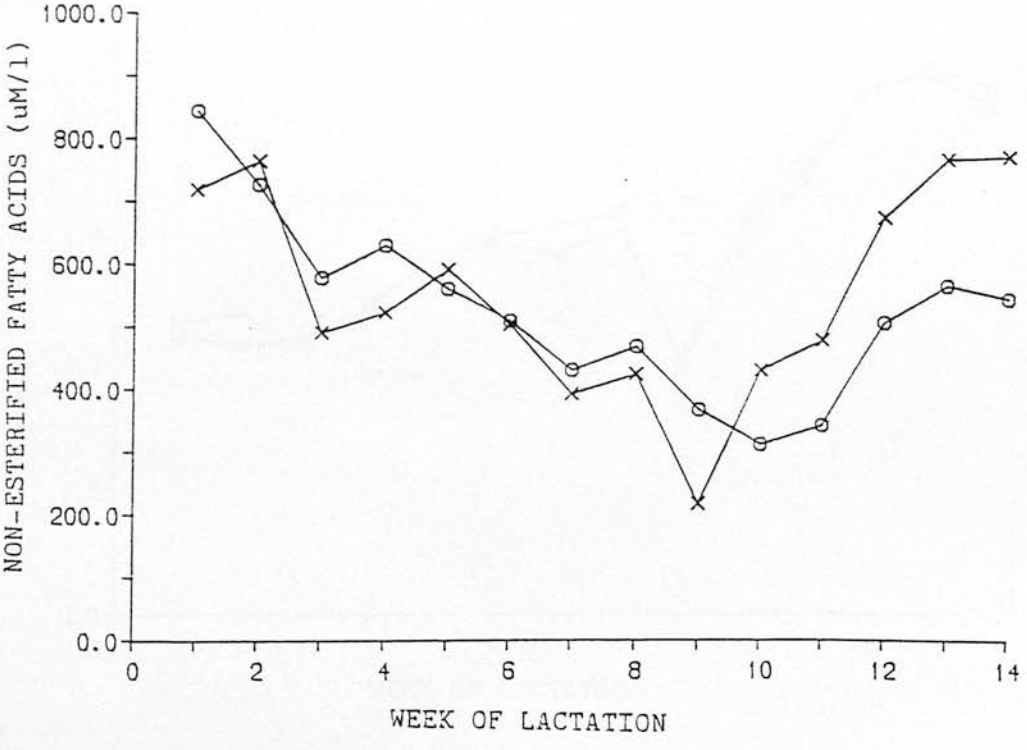


Figure 51. Back-transformed mean plasma 3-OHB concentrations (mM/l) during lactation in EF x——x and SBF ewes o——o (s.e.d. (expressed in log (value + 1) units) weeks 2, 6 and 14 = 0.170, 0.078, 0.019)

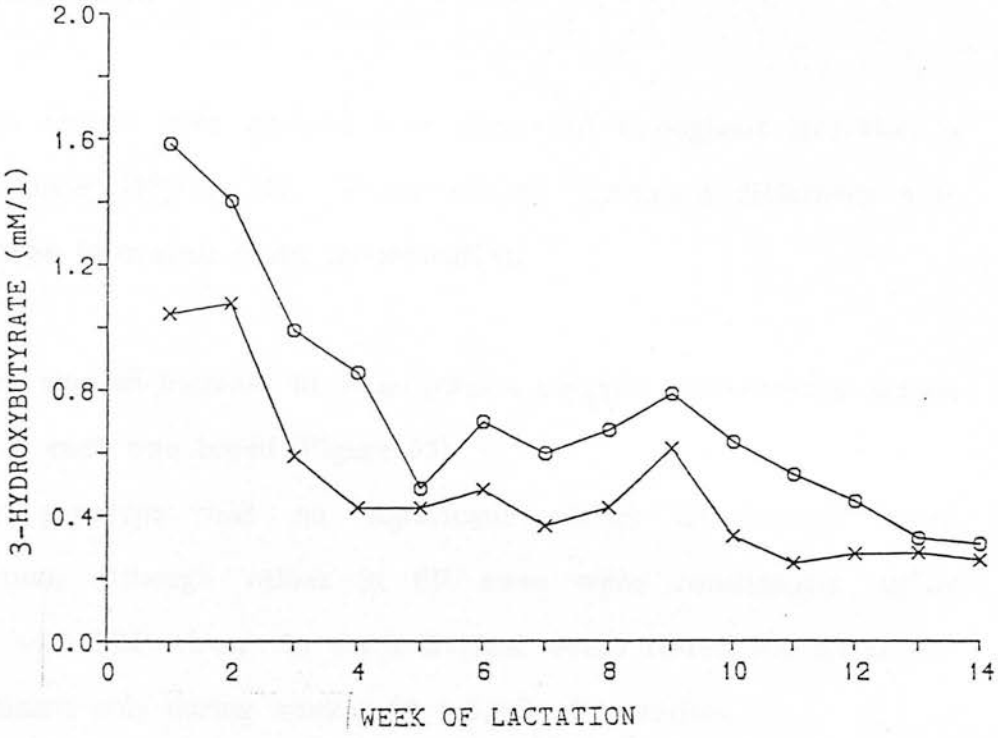
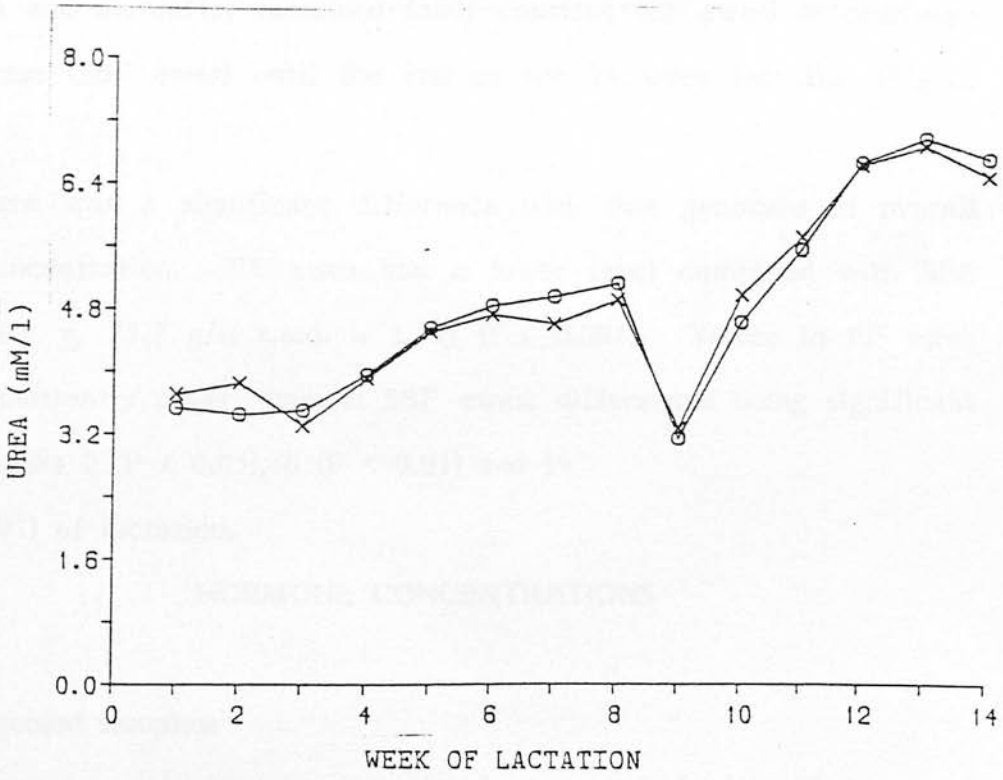


Figure 52. Mean plasma urea concentrations (mM/l) during lactation in EF x——x and SBF ewes o——o (overall s.e.d. = 0.255)



the 3 tested weeks.

Urea

Mean plasma urea concentration increased throughout lactation in each ewe breed (Figure 52). There was no significant difference with ewe genotype in overall mean concentration.

Albumin

There was an increase in mean plasma albumin concentration during lactation in each ewe breed (Figure 53).

Ewe genotype had no significant effect on overall mean concentration, although values in EF ewes were consistently higher compared with SBF ewes. In the individual weeks tested the difference was significant only during week 2 ($P < 0.05$) of lactation.

Protein

Mean plasma protein concentration increased up to week 8 of lactation and thereafter remained fairly constant (EF ewes) or continued to increase (SBF ewes) until the end of the 14 week lactation (Figure 54).

There was a significant difference with ewe genotype in overall mean concentration. EF ewes had a lower level compared with SBF ewes (66.4 *v.* 73.2 g/l; s.e.d. = 1.31; $P < 0.001$). Values in EF ewes were consistently lower than in SBF ewes, differences being significant during weeks 2 ($P < 0.05$), 6 ($P < 0.01$) and 14 ($P < 0.001$) of lactation.

HORMONE CONCENTRATIONS

Insulin

Weekly pooled samples:

Mean plasma insulin concentration increased during the first 4 to 5 weeks of lactation and generally decreased slowly between week 5 and 14 of lactation in each ewe breed (Figure 55).

Figure 53. Mean plasma albumin concentrations (g/l) during lactation in EF x——x and SBF ewes o——o (s.e.d. weeks 2, 6 and 14 = 1.15, 1.13, 1.01)

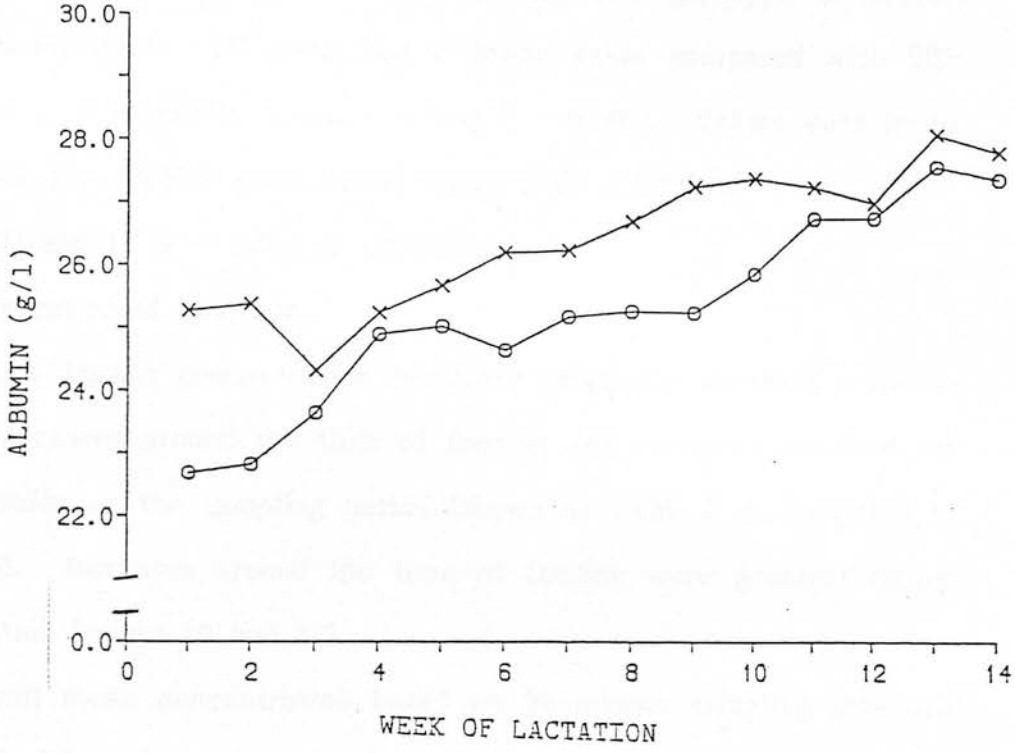
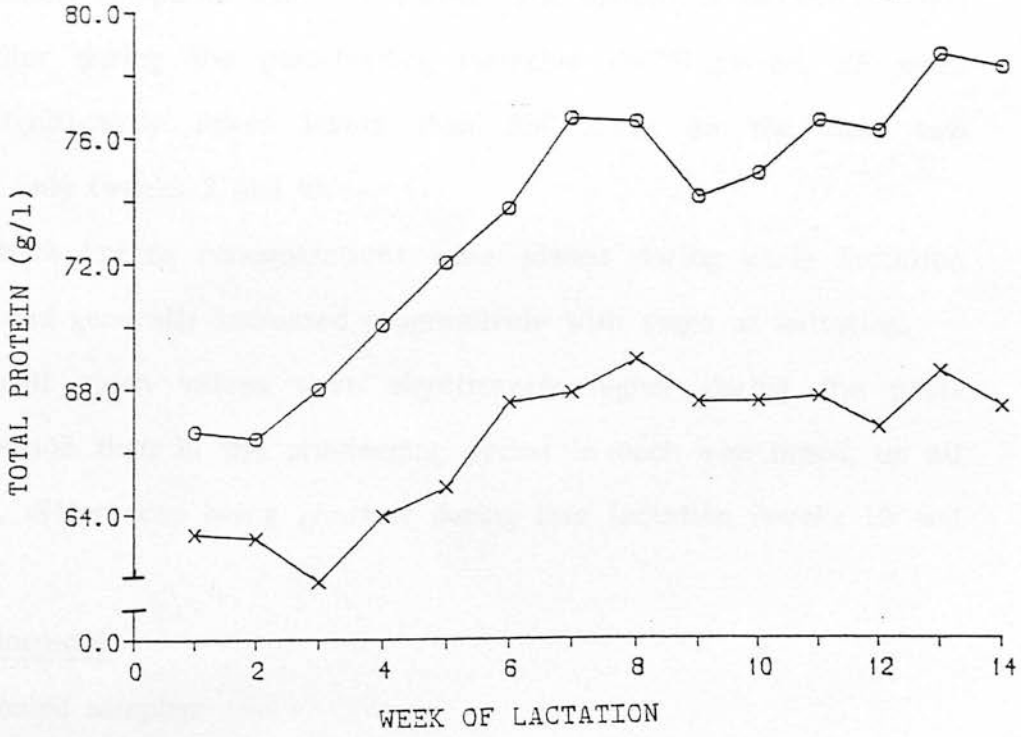


Figure 54. Mean plasma protein concentrations (g/l) during lactation in EF x——x and SBF ewes o——o (s.e.d. weeks 2, 6 and 14 = 1.50, 1.66, 1.66)



There was a significant difference with ewe genotype in overall mean concentration. EF ewes had a lower value compared with SBF ewes (4.36 v. 9.53 mU/l; s.e.d. = 0.994; $P < 0.001$). Values were lower in EF ewes than in SBF ewes during weeks 2 ($P < 0.05$), 6 ($P < 0.001$) and 14 ($P < 0.01$) of lactation.

Frequently collected samples:

Plasma insulin concentration remained relatively constant prior to feeding, increased around the time of feeding and remained elevated for the remainder of the sampling period (shown at week 2 of lactation in Figure 56). Increases around the time of feeding were greatest during late lactation (weeks 10 and 14).

Overall mean concentration based on 20 minute sampling intervals (Table 16) differed significantly between the two genotypes during the pre-feeding period (samples 1-9) on each of the days during lactation when these samples were collected. EF ewes had consistently lower concentrations compared with SBF ewes. The difference between breeds was smaller during the post-feeding (samples 13-25) period, EF ewes having significantly lower levels than SBF ewes on the first two occasions only (weeks 2 and 4).

In both breeds concentrations were lowest during early lactation (week 2) and generally increased progressively with stage of lactation.

Overall mean values were significantly higher during the post-feeding period than in the pre-feeding period in each ewe breed, on all occasions, differences being greatest during late lactation (weeks 10 and 14).

Growth Hormone

Weekly pooled samples:

Mean plasma GH concentration generally declined during lactation in both ewe breeds although there was a marked increase during the first 2 weeks of lactation in EF ewes (Figure 57).

Figure 55. Mean plasma insulin concentrations (mU/l) during lactation in EF x——x and SBF o——o (s.e.d. weeks 2, 6 and 10 = 1.628, 1.447, 1.496)

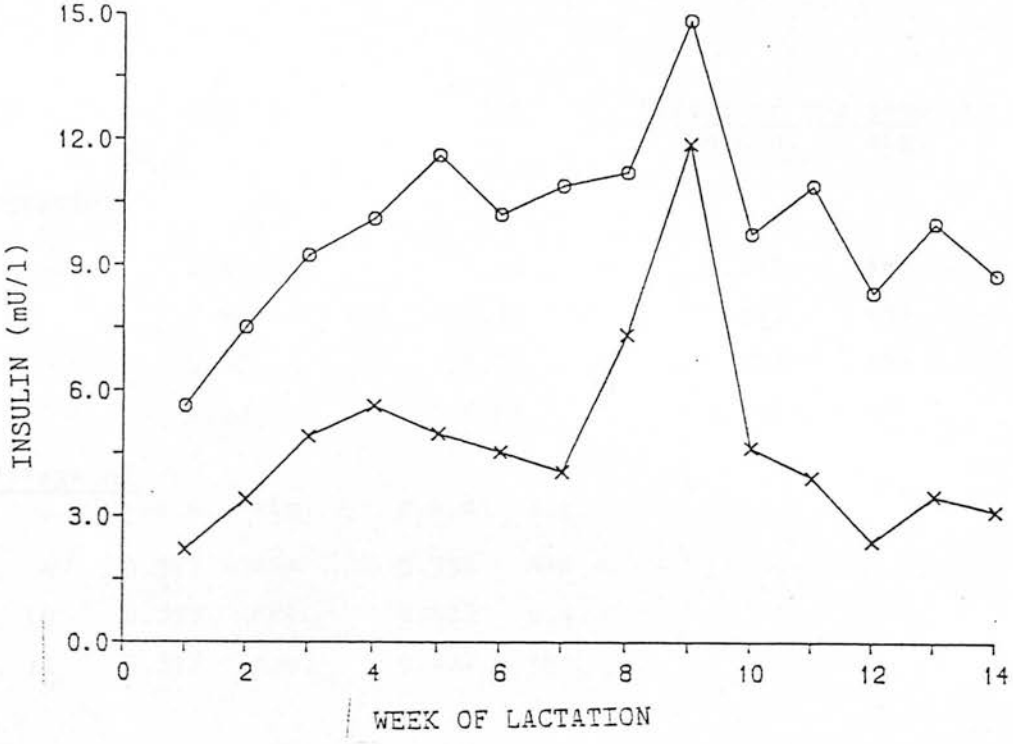


Figure 56. Changes in mean plasma insulin concentration (mU/l) during an 8 hour sampling period at week 2 of lactation in EF x——x and SBF o——o ewes

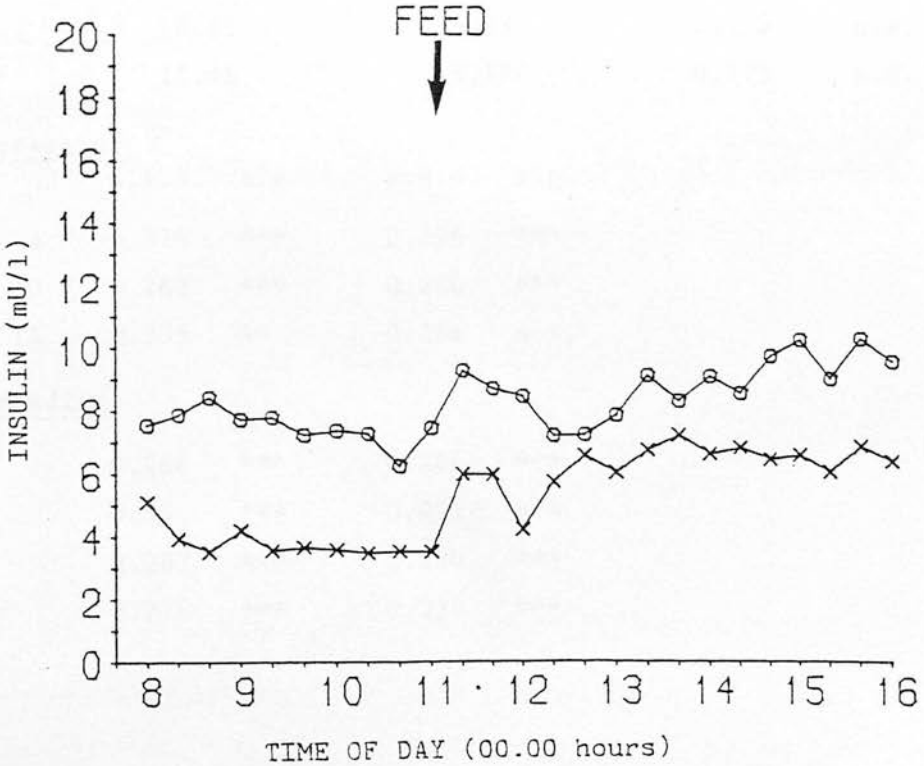


Table 16. Overall mean plasma insulin concentrations (mU/l) during the pre-feeding (samples 1-9) and post-feeding (samples 13-25) periods in EF and SBF ewes during weeks 2, 4, 10 and 14 of lactation.

PRE-FEEDING	EF	SBF	Effect of ewe genotype s.e.d.	sig.
Week of lactation				
2	3.84	7.49	1.277	**
4	5.62	10.38	1.199	***
10	3.90	10.22	1.430	***
14	4.30	9.01	1.350	**

Effect of stage of lactation	s.e.d.	sig.	s.e.d.	sig.
Week 2 v. 4	0.312	***	0.354	***
Week 4 v. 10	0.355	***	0.422	n.s.
Week 10 v. 14	0.317	n.s.	0.437	**

POST-FEEDING	EF	SBF	Effect of ewe genotype s.e.d.	sig.
Week of lactation				
2	6.34	8.83	1.150	*
4	10.49	12.98	1.058	*
10	14.12	15.23	1.133	n.s.
14	15.45	15.17	0.773	n.s.

Effect of stage of lactation	s.e.d.	sig.	s.e.d.	sig.
Week 2 v. 4	0.316	***	0.296	***
Week 4 v. 10	0.262	***	0.286	***
Week 10 v. 14	0.235	**	0.256	n.s.
Effect of feeding				
Week 2	0.266	***	0.264	***
Week 4	0.351	***	0.293	***
Week 10	0.283	***	0.290	***
Week 14	0.274	***	0.330	***

There was no significant difference with breed of ewe in overall mean concentration.

Frequently collected samples:

Mean plasma GH concentration fluctuated throughout the sampling period showing no clear pattern during early lactation (weeks 2 and 4). In late lactation (weeks 10 and 14) levels tended to decline throughout the sampling period (shown at week 2 of lactation in Figure 58).

There were few significant differences with ewe genotype in overall mean concentration (Table 17).

There was a marked and significant decrease in GH concentration with advancing stage of lactation in each ewe breed during both the pre- and post-feeding periods.

Feeding had no consistent effect on overall mean levels during early lactation (week 2) although in the later stages of lactation (weeks 4, 10 and 14) post-feeding levels were, generally, significantly lower compared with pre-feeding levels.

Cortisol

Weekly pooled samples:

There was very little change in mean plasma cortisol concentration throughout lactation in each ewe breed (Figure 59).

EF ewes had a lower overall mean value compared with SBF ewes (5.73 v. 7.59 ug/l; s.e.d. (expressed in log (value+1) units) = 0.105; $P < 0.05$), although in the individual weeks tested differences were significant ($P < 0.01$) only during week 6 of lactation.

Frequently collected samples:

Mean plasma cortisol concentration fluctuated throughout the sampling period (shown at week 2 of lactation in Figure 60).

Overall mean pre- and post-feeding concentrations (Table 18) were lower in EF than SBF ewes at all stages of lactation, although differences were significant during the pre-feeding period only.

Figure 57. Back-transformed mean plasma GH concentrations (ug/l) during lactation in EF x——x and SBF ewes o——o (overall s.e.d. (expressed in log (value + 1) units) = 0.140)

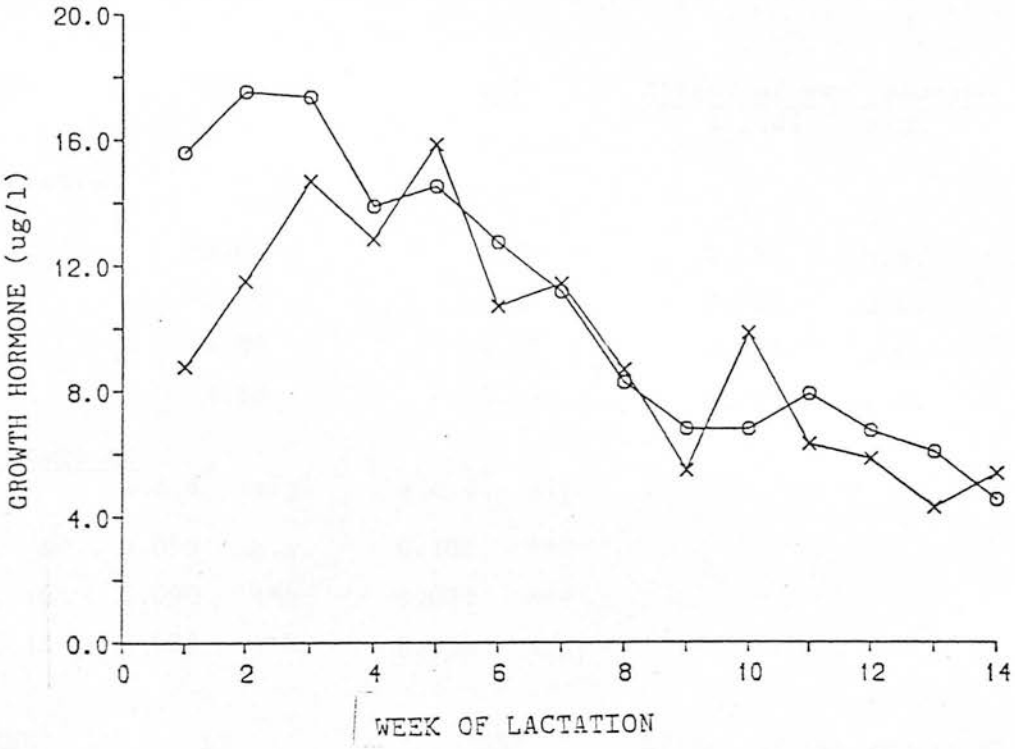


Figure 58. Changes in mean plasma GH concentration (ug/l) during an 8 hour sampling period at week 2[illegible] of lactation in EF x——x and SBF ewes o——o

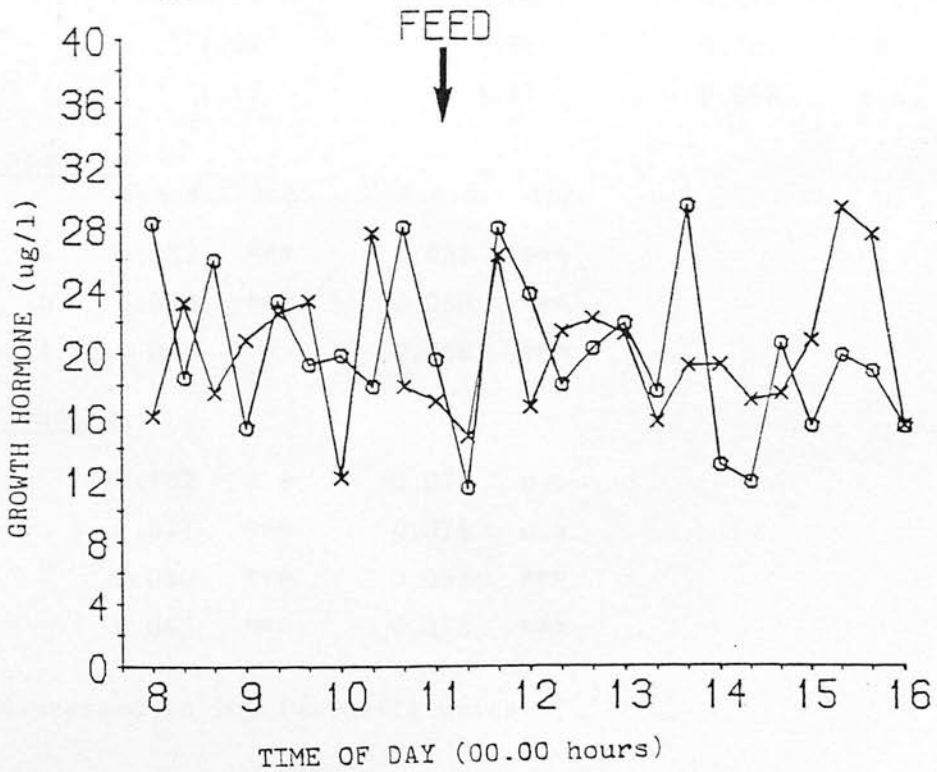


Table 17. Back-transformed overall mean plasma GH concentrations (ug/l) during the pre-feeding (samples 1-9) and post-feeding (samples 13-25) periods in EF and SBF ewes during weeks 2, 4, 10 and 14 of lactation.

PRE-FEEDING	EF	SBF	Effect of ewe genotype s.e.d.+	sig.
Week of lactation				
2	13.66	16.06	0.256	n.s.
4	13.89	9.58	0.199	n.s.
10	4.89	4.49	0.157	n.s.
14	3.38	3.78	0.145	n.s.

Effect of stage of lactation	s.e.d.+	sig.	s.e.d.+	sig.
Week 2 v. 4	0.099	n.s.	0.102	***
Week 4 v. 10	0.090	***	0.071	***
Week 10 v. 14	0.083	***	0.058	n.s.

POST-FEEDING	EF	SBF	Effect of ewe genotype s.e.d.+	sig.
Week of lactation				
2	13.64	14.38	0.258	n.s.
4	5.51	8.60	0.214	n.s.
10	1.01	1.86	0.161	*
14	1.17	1.17	0.088	n.s.

Effect of stage of lactation	s.e.d.+	sig.	s.e.d.+	sig.
Week 2 v. 4	0.083	***	0.087	***
Week 4 v. 10	0.052	***	0.060	***
Week 10 v. 14	0.044	*	0.058	***
Effect of feeding				
Week 2	0.102	n.s.	0.074	n.s.
Week 4	0.071	***	0.075	n.s.
Week 10	0.050	***	0.063	***
Week 14	0.065	***	0.053	***

+s.e.d. expressed in log (value+1) units

Figure 59. Back-transformed mean plasma cortisol concentration (ug/l) during lactation in EF x——x and SBF ewe o——o (s.e.d. (expressed in log (value + 1) units) weeks 2, 6 and 14 = 0.154, 0.112, 0.132)

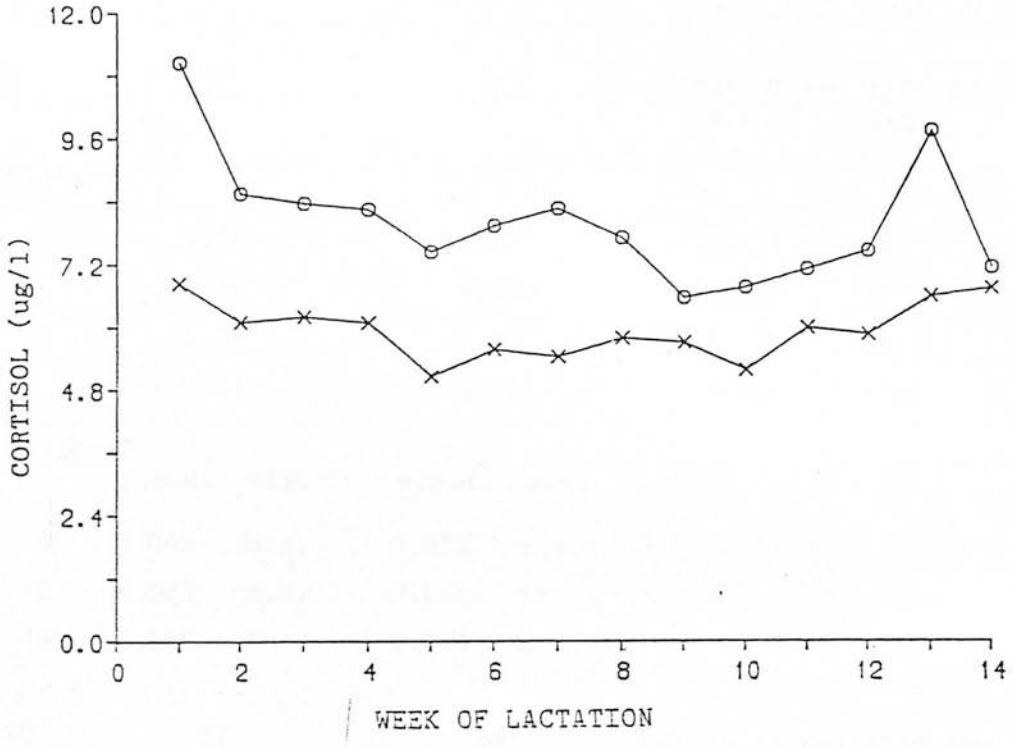


Figure 60. Changes in mean plasma cortisol concentration (ug/l) during an 8 hour sampling period at week 2 of lactation in EF x——x and SBF o——o ewes

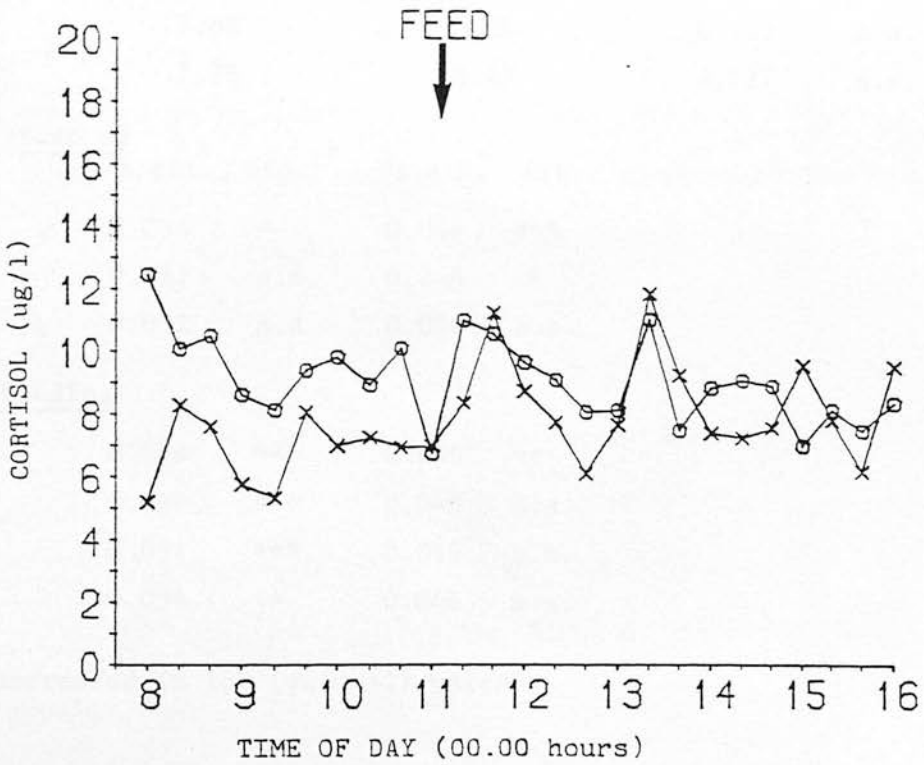


Table 18. Back-transformed overall mean plasma cortisol concentrations (ug/l) during the pre-feeding (samples 1-9) and post-feeding (samples 13-25) periods in EF and SBF ewes during weeks 2, 4, 10 and 14 of lactation.

PRE-FEEDING	EF	SBF	Effect of ewe genotype s.e.d.+	sig.
Week of lactation				
2	6.06	8.87	0.127	*
4	6.34	9.48	0.097	**
10	5.86	8.09	0.123	*
14	6.81	8.17	0.123	n.s.

Effect of stage of lactation	s.e.d.+	sig.	s.e.d.+	sig.
Week 2 v. 4	0.062	n.s.	0.052	n.s.
Week 4 v. 10	0.053	n.s.	0.048	**
Week 10 v. 14	0.049	**	0.046	n.s.

POST-FEEDING	EF	SBF	Effect of ewe genotype s.e.d.+	sig.
Week of lactation				
2	7.29	7.61	0.120	n.s.
4	8.59	9.30	0.112	n.s.
10	7.88	8.30	0.123	n.s.
14	7.76	8.49	0.127	n.s.

Effect of stage of lactation	s.e.d.+	sig.	s.e.d.+	sig.
Week 2 v. 4	0.054	*	0.046	***
Week 4 v. 10	0.052	n.s.	0.044	*
Week 10 v. 14	0.052	n.s.	0.044	n.s.
Effect of feeding				
Week 2	0.059	**	0.048	**
Week 4	0.056	***	0.043	n.s.
Week 10	0.051	***	0.042	n.s.
Week 14	0.054	*	0.046	n.s.

+s.e.d. expressed in log (value+1) units

There were no consistent or marked changes with stage of lactation in overall mean concentration.

Feeding had no consistent effect on overall mean cortisol concentration in SBF ewes, although the post-feeding value was significantly higher compared with the pre-feeding value at all stages of lactation in EF ewes.

Prolactin

Weekly pooled samples:

Changes in plasma prolactin concentration were erratic during early lactation although levels tended to decrease steadily between week 6 and 14 of lactation in each ewe breed (Figure 61).

EF ewes had a lower overall mean value compared with SBF ewes (316.1 v. 457.1 ug/l; s.e.d. (expressed in log units) = 0.17; $P < 0.05$). The values in EF ewes were lower than in SBF ewes during weeks 6 ($P < 0.001$) and 14 ($P < 0.05$) of lactation.

Frequently collected samples:

Mean plasma prolactin concentration generally declined throughout the 8 hour sampling period (shown at week 2 in Figure 62).

Overall mean levels were consistently lower in EF than SBF ewes, although generally differences were significant during the pre-feeding period only (Table 19).

During both the pre- and post-feeding periods there was a consistent and generally significant increase in level during early lactation (i.e. between week 2 and 4) followed by a progressive decrease over the last 2 stages of lactation in each ewe breed.

Levels were generally lower following feeding in each ewe breed.

Triiodothyronine

Weekly pooled samples:

There was an increase in mean T^3 concentration during the first

Figure 61. Back-transformed mean plasma prolactin concentrations (ug/l) during lactation in EF x——x and SBF ewes o——o (s.e.d. (expressed in log units) weeks 2, 6 and 14 = 0.321, 0.118, 0.245)

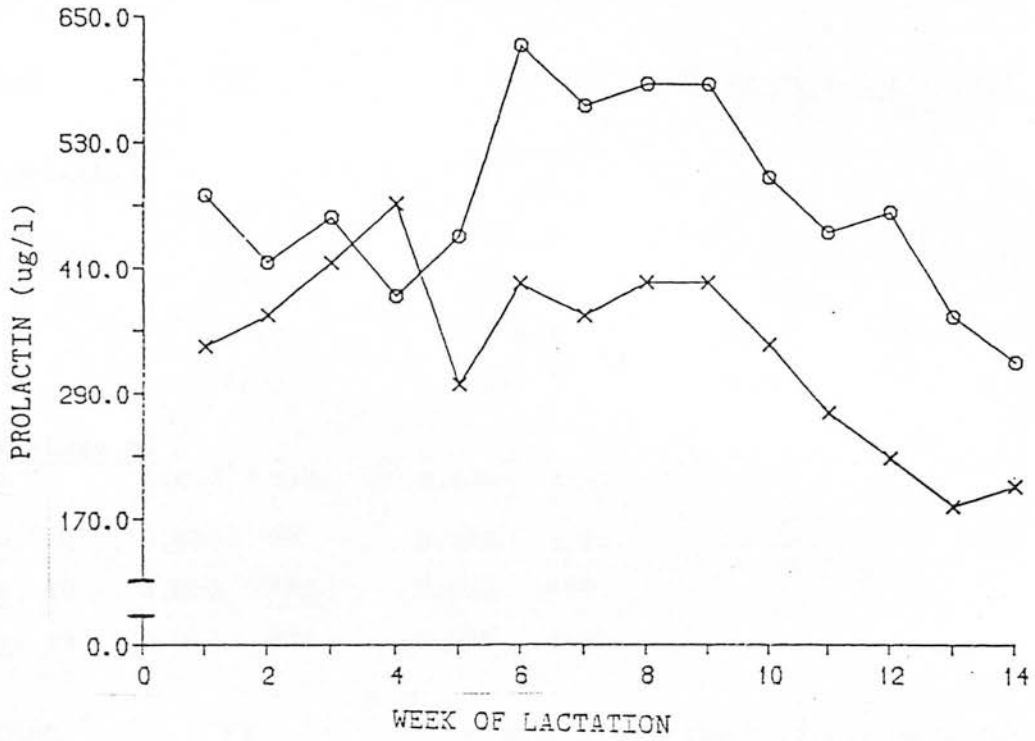


Figure 62. Changes in mean plasma prolactin concentration (ug/l) during an 8 hour sampling period at week 2 of lactation in EF x——x and SBF ewes o——o

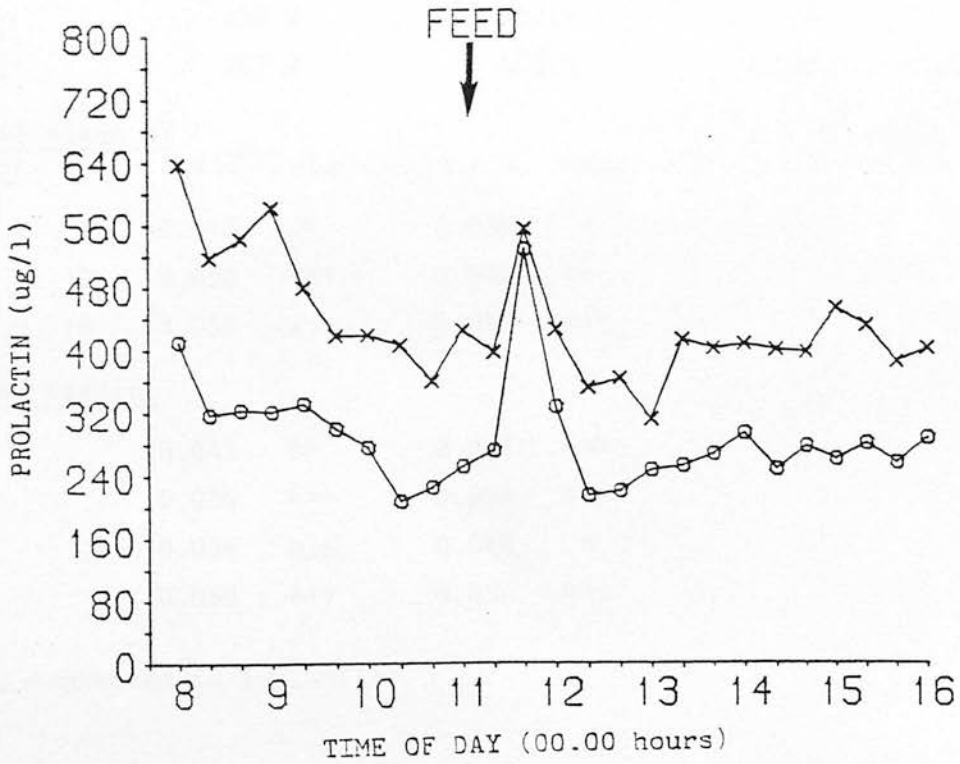


Table 19. Back-transformed overall mean plasma prolactin concentrations (ug/l) during the pre-feeding (samples 1-9) and post-feeding (samples 13-25) periods in EF and SBF ewes during weeks 2, 4, 10 and 14 of lactation.

PRE-FEEDING	EF	SBF	Effect of ewe genotype s.e.d.+	sig.
Week of lactation				
2	356.7	536.5	0.201	n.s.
4	443.6	557.8	0.070	**
10	213.8	396.2	0.192	**
14	171.2	354.2	0.232	**

Effect of stage of lactation	s.e.d.+	sig.	s.e.d.+	sig.
Week 2 v. 4	0.068	**	0.034	n.s.
Week 4 v. 10	0.060	***	0.051	***
Week 10 v. 14	0.042	***	0.056	n.s.

POST-FEEDING	EF	SBF	Effect of ewe genotype s.e.d.+	sig.
Week of lactation				
2	315.4	368.0	0.244	n.s.
4	355.3	400.6	0.110	n.s.
10	229.3	358.9	0.128	**
14	207.9	268.5	0.125	n.s.

Effect of stage of lactation	s.e.d.+	sig.	s.e.d.+	sig.
Week 2 v. 4	0.047	*	0.038	*
Week 4 v. 10	0.052	***	0.040	**
Week 10 v. 14	0.054	n.s.	0.037	***
Effect of feeding				
Week 2	0.045	**	0.045	***
Week 4	0.036	***	0.034	***
Week 10	0.056	n.s.	0.046	*
Week 14	0.059	***	0.034	***

+s.e.d. expressed in log units

half of lactation, followed by a decrease during the second half of lactation in each ewe breed (Figure 55).

EF ewes had a significantly lower overall mean value than SBF ewes (1.12 v. 1.32 ug/l; s.e.d. = 0.061; $P < 0.01$). Levels were consistently lower in EF compared with SBF ewes and differences were significant during weeks 2 ($P < 0.05$) and 14 ($P < 0.001$) of lactation.

Thyroxine

Weekly pooled samples:

Mean plasma T^4 concentration generally increased during lactation in each ewe breed (Figure 64).

There was no significant difference with ewe genotype in overall mean concentration. Values were consistently lower in EF ewes compared with SBF ewes during the final 4 weeks of the 14 week lactation study.

Figure 63. Mean plasma T^3 concentrations (ug/l) during lactation in EF x——x and SBF ewes o——o (s.e.d. weeks 2, 6 and 14 = 0.087, 0.098, 0.084)

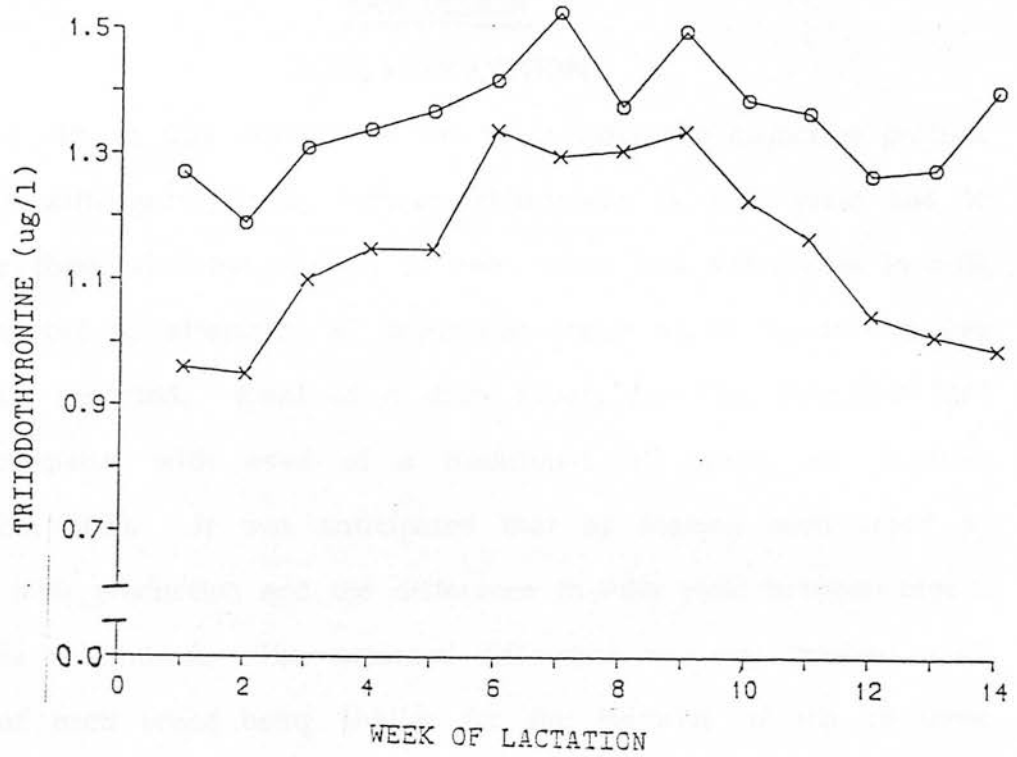
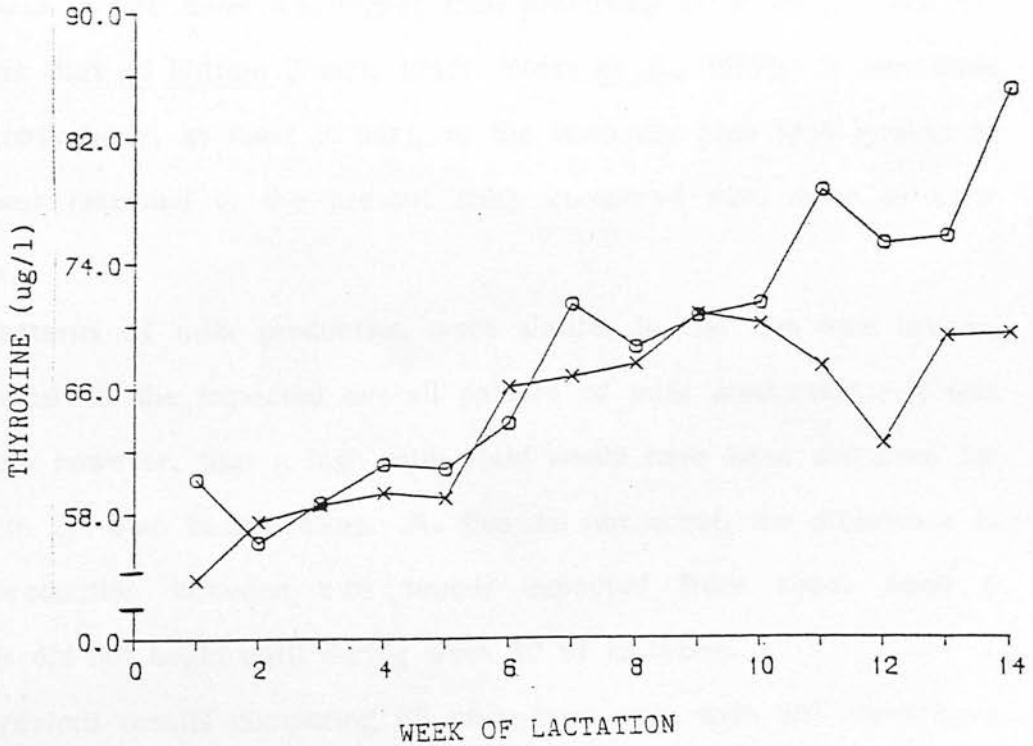


Figure 64. Mean plasma T^4 concentrations (ug/l) during lactation in EF x——x and SBF ewes o——o (overall s.e.d. = 3.98)



DISCUSSION

MILK PRODUCTION

The aim of this experiment was to compare the endocrine profiles of ewes with genotypically induced differences in milk yield and to compare them with the profiles of ewes which had differences in milk yield created by alteration of nutritional status as in the two studies previously reported. Ewes of a dairy breed, the East Friesland (EF) were compared with ewes of a traditional hill breed, the Scottish Blackface (SBF). It was anticipated that by feeding each breed ad libitum milk production and the difference in milk yield between breeds would be maximised. The expected difference was not realised, milk yields of each breed being similar for the majority of the 14 week lactation study. Milk production in EF ewes was lower than previously recorded for dairy ewes (Peart, 1973); it may have been limited in the present study by the appetite of the pure-bred EF lambs. In contrast milk yield in SBF ewes was higher than previously recorded in ewes fed a similar diet ad libitum (Peart, 1968; Peart et al., 1979); it may have been attributable, at least in part, to the unusually high feed intakes in SBF ewes recorded in the present study compared with those in their studies.

Patterns of milk production were similar in the two ewe breeds, and similar to the expected overall pattern of milk production. It was expected, however, that a high milk yield would have been sustained for longer in EF than in SBF ewes. As this did not occur, the difference in milk production between ewe breeds expected from about week 6 onwards did not begin until during week 10 of lactation.

Previous results comparing EF cross-bred ewes with SBF ewes have shown that milk fat content is lower in the former breed while milk protein and lactose content is unaffected by ewe genotype (Doney et al., 1981; Doney et al., 1983). The results of the present study are similar.

Despite the lack of differences in milk production between ewe breeds an examination of circulating blood metabolite and hormone status during lactation is of value in establishing whether or not milk production in different genotypes is influenced by similar endocrine factors and whether or not genotype and nutritional effects on milk yield are mediated through the same endocrine factors.

NUTRITIONAL STATUS

Energy status

In the present study ewes of each breed were fed the same diet, although intakes were generally slightly higher in EF than in SBF ewes during lactation. Although consistent gains in live weight in each ewe breed during virtually the whole lactation period, by themselves, suggest that nutrient intake was in excess of requirements for maintenance and milk production, the reduction in body condition score during early lactation in each ewe breed, indicates that a small amount of tissue mobilisation occurred during this period. This conclusion is corroborated by elevated NEFA and 3-OHB levels during early lactation. Increases in body condition during mid-lactation in each ewe breed and declining NEFA and 3-OHB levels suggest that nutrients were in excess of requirements by this stage of lactation. The similarity between rates of live weight gain between ewe breeds implies that rate of body tissue accretion was also similar. Rate of condition score gain was slightly lower in EF compared with SBF ewes but as the distribution of adipose tissue differs between these breeds (Butler-Hogg and Whelehan, 1986) this may be misleading.

It is unlikely that the increase in NEFA levels during the latter half of lactation is related to mobilisation of adipose tissue as all other indices suggest that ewes of both breeds were in positive energy balance during this period. It is possible that the increased level of circulating NEFA may be a function of the release of fatty acids from the

mammary gland (Annison et al., 1967), at a time when milk fat production is declining and changes in amount and/or rate of adipose tissue turnover.

Protein status

It is also unlikely that the observed breed differences in plasma albumin and total protein concentrations are attributable to differences in protein status as all ewes were adequately fed during lactation, and milk protein production was similar in each breed throughout lactation. Increasing urea, albumin and protein levels with advancing stage of lactation probably reflect an increase in amino acid availability as milk yield decreased and nutrient intake was maximised.

In summary, the observed patterns of feed intake, milk production and the perceived patterns of nutrient partitioning (to and from body tissues) were similar in each ewe breed. The biological significance of breed differences in some of the blood metabolite levels is not known but may be indicative of breed differences in metabolism and its endocrine control.

HORMONE STATUS

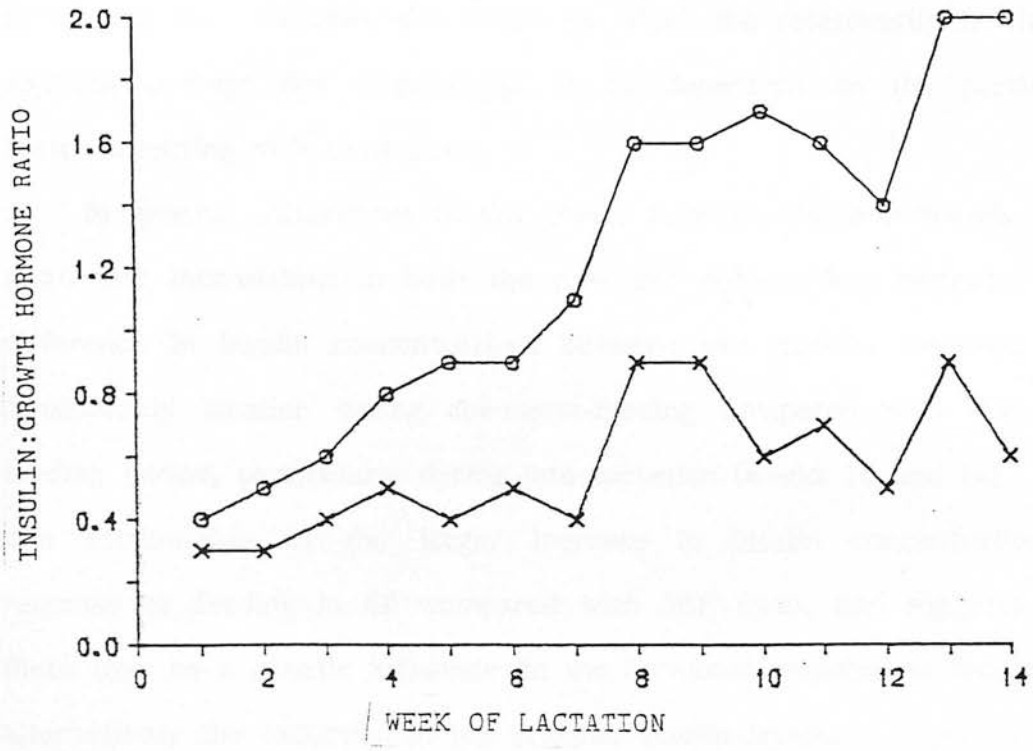
Weekly GH levels were similar for ewes of each breed and changes in GH levels throughout lactation reflected changes in milk production. This is consistent with the suggested role of GH in the control of milk production (Cowie et al., 1980; Trenkle, 1981). A superficial interpretation of the results might suggest that the difference in weekly insulin concentration associated with ewe genotype would be related to a difference in the amount of nutrients being directed towards body tissue between ewe breeds. This idea is clearly not supported by the observed live weight and body condition changes throughout lactation in each ewe breed. It is also noteworthy that the difference in insulin concentration between ewe breeds, despite apparent similarities in nutrient metabolism,

contrasts with previous work by Hart (1983) who observed similar insulin values in different cow genotypes fed to achieve similar live weight changes during lactation. It must be borne in mind, however, that live weight change is a crude measure of nutrient status. Furthermore this result illustrates the limitations of examining one hormone in isolation; it also suggests that differences in hormone level may have different biological significance according to breed.

The lower insulin level in EF compared with SBF ewes was associated with a lower milk fat content in these ewes. This agrees with results of studies in cattle in which exogenous insulin administration resulted in higher milk fat content compared with untreated animals (Cowie et al., 1980). However, in contrast an increase in the amount of concentrate fed as a proportion of the whole diet generally results in a reduction in milk fat content and is thought to be related to an increase in circulating insulin levels (Jenny, Polan and Thyle, 1974). Obviously milk fat content is not dependent on insulin levels alone and so the relationship between insulin and milk fat levels differs with nutritional and other circumstances.

A consequence of the lower insulin level in EF compared with SBF ewes was the lower insulin:GH ratio in the former breed throughout lactation (Figure 65). The fact that this was not associated with any apparent increase in nutrient availability to the mammary gland, again suggests that there are differences in the mechanisms controlling milk production in the two breeds. The insulin:GH ratio increased during lactation in each ewe breed, as in the previous study, which is consistent with the increasing direction of nutrients towards body tissue, however, in contrast to results of the previous experiment the change in the insulin:GH ratio throughout lactation was as a consequence of decreasing GH concentration and fairly constant insulin concentration throughout lactation.

Figure 65. Mean plasma insulin : geometric GH ratio during lactation in EF x——x and SBF o——o ewes.



The relationship between insulin and GH seems to be a key factor in the control of nutrient partitioning during lactation in all the studies in this series. However, the means by which the relationship is altered appears, perhaps not surprisingly, to be dependent on the particular factor affecting milk production.

In general differences in GH levels between the ewe breeds were small and inconsistent in both the pre- and post-feeding periods. The difference in insulin concentrations between ewe breeds, however, was considerably smaller during the post-feeding compared with the pre-feeding period, particularly during late lactation (weeks 10 and 14). This was attributable to the larger increase in insulin concentration in response to feeding in EF compared with SBF ewes, and suggests that there may be a genetic influence on the hormonal response to feeding or alternatively the reduction in pre prandial insulin levels.

There was a marked increase in the size of the insulin response to feeding with advancing stage of lactation in each ewe breed. This may be related, at least in part, to the increase in feed intake during lactation; however, because the ewes in the present study were fed ad libitum it is unlikely that there would be a large post prandial increase in circulating nutrient levels and the increase in insulin following feeding is a neurological response rather than a function of a nutrient surge (Weekes and Godden, 1981). Another possibility is that the insulin response to feeding may have been suppressed during early lactation; Lomax et al. (1979) suggested that the insulin response to feeding was suppressed in lactating compared with non-lactating cows.

The decline in pre-feeding GH levels with advancing stage of lactation contrasts with results of the previous studies in which pre-feeding GH levels remained fairly constant throughout lactation. This may be a function of the different feeding regimes in each case. In the

present study ewes were fed ad libitum and unlike ewes fed on the restricted feeding regime in the two previous studies would not have been subject to a short-term pre prandial energy deficit. Thus GH levels may not necessarily have been elevated during the pre-feeding period.

As in the case of insulin, cortisol levels were also lower in EF compared with SBF ewes and this could have affected the rate of gluconeogenesis, EF ewes having a lower rate compared with SBF ewes. However, there is no real evidence which supports this. In fact plasma glucose levels were higher in EF than SBF ewes which implies glucose availability was greater in the former ewe, although the possibility that glucose turnover rates may be different between ewe breeds cannot be excluded. It is also highly unlikely that the difference in cortisol levels was associated with differences in protein catabolism as all ewes were adequately fed throughout most of the lactation period.

The fact that the difference in cortisol concentration associated with ewe genotype was not observed during the post-feeding period is related to the increase in the size of the cortisol response to feeding in EF compared with SBF ewes, although the reason for the difference in this response is not clear.

Prolactin levels generally reflected the pattern of milk production in each ewe breed, although circulating prolactin levels were unrelated to milk production.

As in the case of cortisol, differences with ewe genotype in prolactin concentration following feeding, were generally not significant. It is not clear whether the change in circulating prolactin levels was merely a function of the pre prandial difference in prolactin concentration or a difference in the physiological response to feeding.

Although in general there was no difference with ewe genotype in mean T4 levels, levels of the more biologically active thyroid hormone T3 were significantly lower in EF than in SBF ewes. It is noteworthy that the divergence in T4 levels towards the end of lactation was associated with a difference in milk production between ewe breeds at this time; levels being lower in ewes yielding the highest quantity of milk.

As previously indicated interpretation of these data require a more complete knowledge of T^4 to T^3 conversion rate and of the interactions of the thyroid hormones and other hormones.

CONCLUSION

The results of the breed comparison provide a further insight into the complexities of the hormonal control of milk production and illustrate the difficulties of interpretation of hormones in isolation. The results suggest that genetic differences in hormone levels exist which are independent of circulating nutrient levels. As in previous experiments the insulin:GH ratio seems to be an important factor in the control of milk production. However, changes in this ratio were not a function of the same hormonal changes in all experiments.

CHAPTER 7

GENERAL DISCUSSION

INTRODUCTION

In the series of experiments described the aim of the work was to alter milk production levels in lactating ewes by manipulating three different factors known to affect the ewes physiological state and level of milk production, although not necessarily by the same endocrine mechanisms (Figure 66). By this means it was intended to examine the relationships between the levels of milk production and associated endocrine status with the objective of increasing understanding of the endocrine factors controlling the partitioning of nutrients between the mammary gland and body tissue, an important factor in the control of milk production, and to determine whether or not the different manipulations affected nutrient partitioning and milk production through changes in the same or different endocrine mechanisms.

As an aid to the discussion of the probable effects of insulin, GH, cortisol, prolactin and the thyroid hormones on aspects of glucose, adipose tissue and protein tissue metabolism are summarised in Figure 67, which is not intended to be either exhaustive or detailed.

Level of milk production and milk composition are principally influenced by the amount of nutrients supplied to the mammary gland. Factors affecting nutrient availability are numerous, but almost all are either endocrine in nature or under endocrine control. It is impossible to identify a single driving force behind the control of milk production as circulating nutrient levels and endocrine factors are in a constant state of equilibrium, regardless of the level of milk production and it is the point of equilibrium which changes according to the stimulus to milk production, physiological status and perhaps ewe genotype. It is quite conceivable that the same point of equilibrium may be reached by

Figure 66. Relationship between factors known to stimulate milk production (i.e. suckling stimulus, dietary protein intake and ewe genotype), nutriet flow and hormone status.

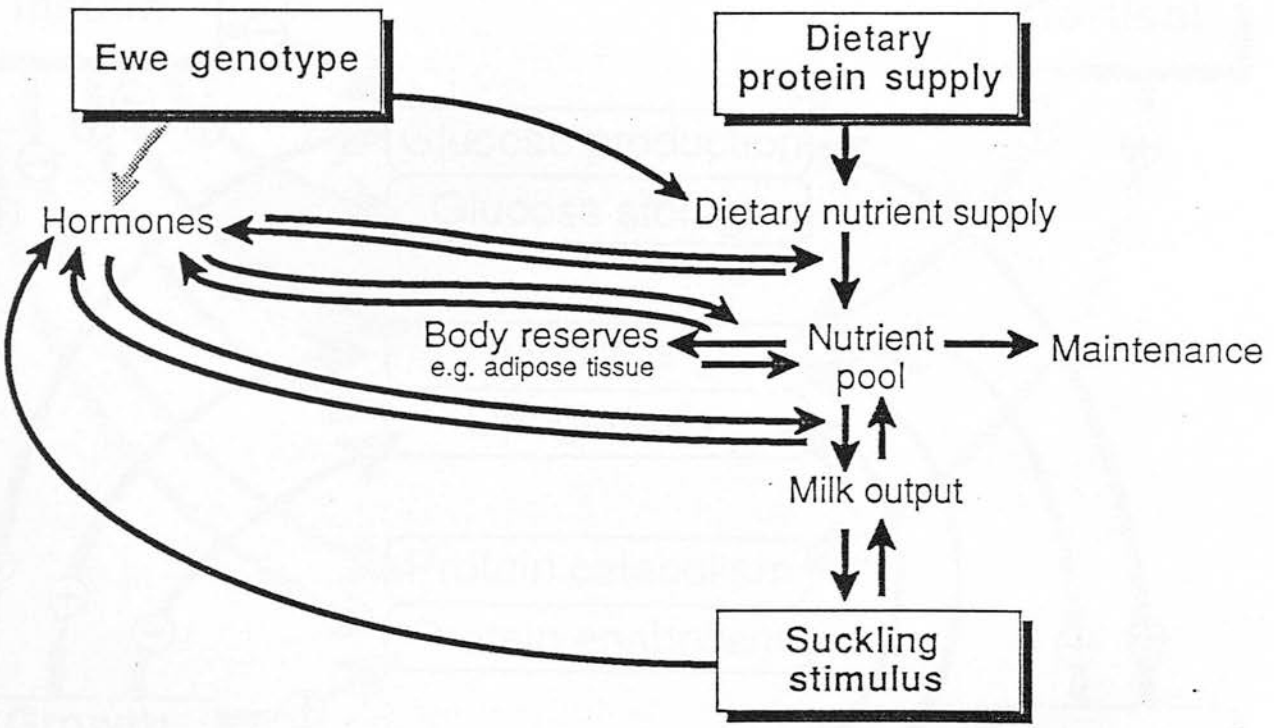
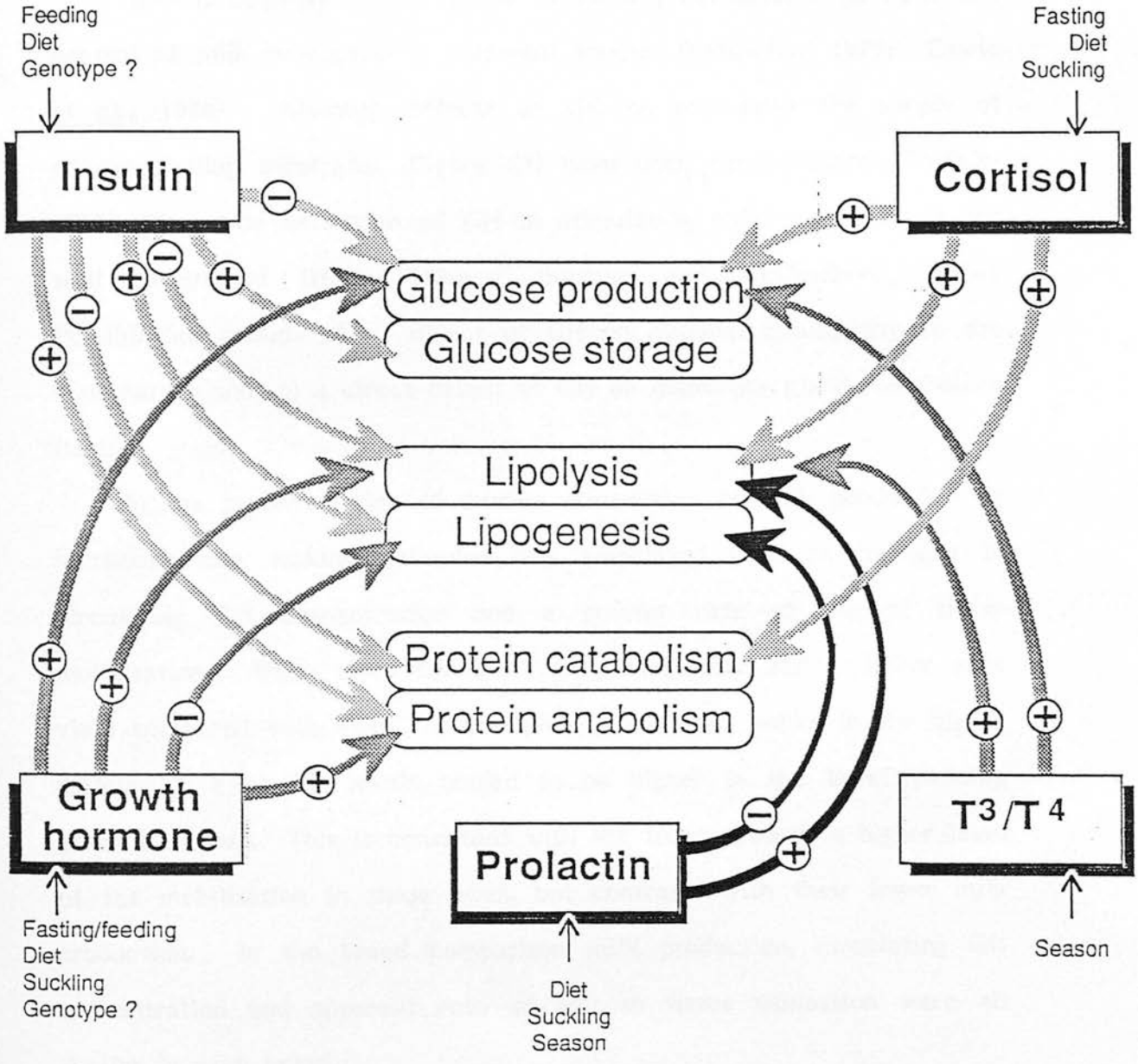


Figure 67. Some factors affecting insulin, GH, cortisol, prolactin and thyroid hormone concentrations and some of the probable effects of these hormones on aspects of glucose, adipose tissue and protein tissue metabolism.



different changes in hormone status, according to circumstances.

GH - A KEY HORMONE IN THE CONTROL OF MILK PRODUCTION

GH and level of milk production

GH has been identified as one of the key hormones involved in the control of milk production in ruminant species (Fulkerson, 1979; Cowie et al., 1980). Although effects of GH on increasing the supply of energy-yielding substrates (Figure 67) have been demonstrated (Trenkle, 1981), the mode of action of GH in stimulating milk production is not well understood (Hart, 1983; Bauman and McCutcheon, 1986). Possibilities include a) an effect of GH on nutrient availability to the mammary gland, b) a direct effect of GH on mammary gland metabolism itself.

In the present series of studies stimulation of milk production by increasing the suckling stimulus was associated with an increase in circulating GH concentration and a greater rate of adipose tissue mobilisation. While ewes fed the high protein diet had a higher milk yield compared with ewes fed the low protein diet, unlike in the higher yielding T ewes GH levels tended to be higher in the lower yielding group (L ewes). This is consistent with the trend towards a higher level of fat mobilisation in these ewes, but contrasts with their lower milk production. In the breed comparison milk production, circulating GH concentration and apparent rate of adipose tissue utilisation were all similar in each breed.

In general overall GH levels were broadly related to the rate of adipose tissue mobilisation which is consistent with a role for GH in increasing energy substrates availability for milk production (Trenkle, 1981; Hart, 1983; Bauman and McCrutcheon, 1986). However, levels were not necessarily related to the level of milk production indicating that GH alone is probably not a driving force of milk production.

GH and change in milk production

In the litter size and dietary protein comparisons weekly GH levels did not alter with stage of lactation despite a marked decline in the milk production and rate of adipose tissue mobilisation in each of the studies throughout lactation. In the genotype comparison, on the other hand, GH levels decreased progressively with advancing stage of lactation in each of the ewe breeds thus reflecting the pattern of milk production and change in degree of adipose tissue utilisation, as estimated by NEFA and 3-OHB levels, during lactation. This suggests that if GH is involved directly in the control of milk production (by increasing nutrient availability or otherwise) then its effects may be confined to the early stages of lactation as GH levels during the latter half of lactation were not related to milk yield in either the litter size or dietary protein comparisons.

Therefore, profiles were not only inconsistent with level of milk production, but were also not always consistent with changes in milk production and rate of adipose tissue mobilisation with stage of lactation. In the litter size and dietary protein comparisons, however, energy status was measured in the preprandial period. It is not unreasonable to assume that GH levels would be elevated during this period (Gow, McDowell and Annison, 1981), when nutrient supply from the diet is absent. In contrast ewes in the breed comparison were fed ad libitum and therefore the preprandial increase in GH concentration, associated with short-term nutrient deficit, was probably smaller. The preprandial increase in GH levels in both the litter size and dietary protein comparisons during late lactation may have masked changes in GH levels associated with stage of lactation such as those recorded in the genotype comparison.

In all studies GH levels during the post-feeding period, tended to decrease with stage of lactation. This would be consistent with a decrease in the requirement for a catabolic or 'anti-anabolic' influence following feeding at stage of lactation when nutrient requirements for milk production were decreasing.

While changes in GH may drive changes in milk production and associated adipose tissue utilisation, differences in the size of changes in GH at feeding may also be involved in the control of nutrient availability to the mammary gland.

Short-term actions of GH

The GH response to increased nutrient availability following feeding was examined in order to determine whether short-term actions of GH during the immediate postprandial period were important in relation to control of milk production.

In the litter size comparison there was a tendency for overall GH levels to increase following feeding. This effect was much more apparent in the dietary protein comparison but was confined in this experiment to early lactation only. In the breed comparison there was no such increase in GH levels following feeding. This increase in GH levels following feeding is contrary to many previous reports in the literature examining the effects of feeding on GH levels on ruminant species (Trenkle, 1971; Trenkle, 1978). While the physiological stimulus responsible for the increase is not clear, the effect is likely to be an increased nutrient availablity at a time when nutrient supply from food and nutrient demand for milk production is maximal.

Role of GH:conclusions

While differences in GH levels were not always related to treatment differences in milk production, values were related to rate of adipose tissue utilisation. Changes in level of milk production and rate

of adipose tissue utilisation as lactation progressed were both related to changes in post-prandial GH levels suggesting that increases in GH concentration following feeding may have an important role in the control of nutrient supply to the mammary gland.

While these obserservations increase knowledge and understanding of the role of GH in the control of milk production, the factors which stimulate GH release and its mode of action remain unclear. Knowledge of changes in GH receptor sites at target tissues throughout lactation is also required.

THE ROLE OF INSULIN IN CONJUNCTION WITH GH

One of the roles of GH is to increase availability of energy providing substrates; however, it also stimulates the deposition of protein (Trenkle, 1981). Insulin, on the other hand, decreases availability of energy providing substrates while stimulating protein deposition (Trenkle, 1981). Thus the effects of altered GH concentrations cannot be assessed without reference to the role of insulin; neither can changes in GH and insulin associated with alterations in energy metabolism be divorced from effects on protein metabolism (Figure 67).

Higher milk yields associated with multiple litters or a high protein diet were associated with a lower insulin:GH ratio during lactation. This would favour mobilisation rather than accretion of nutrients. In the genotype comparison, however, EF ewes had a lower insulin:GH ratio compared with SBF ewes and this was not associated with any difference in either milk production or degree of adipose tissue utilisation.

In ewes suckling either single or twin lambs and in ewes fed the low protein diet, there was no marked change in the weekly insulin:GH ratio with stage of lactation. However, there was a marked increase in the weekly insulin:GH ratio during lactation in each ewe breed in the genotype comparison and to a lesser extent in ewes fed the high protein

diet. However, in contrast to ewes fed the high protein diet, in the genotype study the change was brought about by a decrease in GH level in relation to fairly constant insulin level as opposed to an increase in insulin levels in conjunction with fairly constant GH levels. Furthermore there was a marked increase in the insulin:GH ratio during the post-prandial period in all experiments. It seems likely that changes in the insulin:GH ratio are responsible, at least in part, for the switch in nutrient partitioning from milk to body tissue, although the manner in which the change in ratio is achieved may vary according to circumstances.

Short-term changes in insulin concentration

In all three studies there was a marked increase in insulin levels in response to feeding at all stages of lactation. In general this increase was greater during late lactation compared with early lactation which suggests that the stimulus to deposit nutrients into body stores increased with stage of lactation. The concurrent increase in GH levels following feeding, which was observed during early lactation particularly in the dietary protein comparison, probably serves to balance the effects of insulin on nutrient partitioning and thus maintain the availability of nutrients to the mammary gland.

It is concluded that the relationship between insulin and GH is a key factor in the control of nutrient partitioning towards milk production or body tissue stores during lactation but the means by which the ratio is altered is dependent on the factor affecting milk production. Furthermore, changes in hormone concentration with treatment may be masked by pre-prandial changes in circulating hormone levels.

It is interesting to note that although reduced insulin levels were not always associated with increased milk production they were always associated with lower milk fat content. It is possible that in addition to

its anabolic properties on body tissue, insulin may also have a direct effect on the uptake of milk fat precursors or synthesis of milk fat at the mammary gland.

THE RELATIONSHIP BETWEEN INSULIN AND CORTISOL

Another hormone affecting the supply of both energy and protein-yielding nutrients is cortisol (Trenkle, 1981). Consequently the action of insulin needs to be assessed in relation to that of cortisol as the actions of insulin directly oppose the known actions of cortisol (Figure 67) (Trenkle, 1981).

The higher milk yield associated with increased litter size was associated with a lower insulin:cortisol ratio throughout lactation. The ratio was also lower in the higher yielding high protein group of ewes compared with those fed the low protein diet during early lactation. Insulin:cortisol ratios, like milk yield were similar in the two ewe genotypes. A decrease in this ratio would favour increased availability of nutrients to the mammary gland in the higher yielding group.

It is concluded that the insulin:cortisol ratio generally reflects the perceived differences between treatment groups in nutrient availability. However, changes in the weekly insulin:cortisol ratio did not reflect changes in level of milk production or associated nutrient metabolism. Although the postprandial increase in insulin levels in relation to fairly constant cortisol levels with advancing stage of lactation would favour the deposition of nutrients towards body stores.

THE RELATIONSHIP BETWEEN CORTISOL AND GH

The absence of any changes in cortisol with time suggests that cortisol alone is not involved with changes in milk production associated with stage of lactation; changes in the cortisol:GH ratio are mainly a function of changes in GH, which have been discussed earlier. Treatment differences in the cortisol:GH ratio in the litter size

comparison are consistent with an increase in availability of nutrients for milk production although the physiological reasons for treatment differences in cortisol:GH ratio which occurred in the genotype study remain unclear.

PROLACTIN - DOES IT HAVE A ROLE IN THE CONTROL OF MILK PRODUCTION?

GH, insulin and cortisol operate against a background of prolactin and thyroid hormone concentrations which differed with treatments and season.

In both the litter size and dietary protein comparisons differences in the level of milk production were not associated with differences in plasma prolactin levels. In the breed comparison and seasonal comparison widely different prolactin levels were associated with similar levels of milk production. These results all suggest that prolactin concentration is not a primary determinant of level of milk production.

In all the experiments, major changes in prolactin levels during lactation can probably best be explained in terms of the response of prolactin to a combination of intensity of suckling stimulus and time of year.

The results of the present study strongly suggest that prolactin is unlikely to be involved in the control of level of milk production per se. However, while there was no indication that prolactin affected the supply of nutrients for milk production, the possibility remains that low levels of prolactin may be necessary for continuation of milk synthesis (Fulkerson, 1979). It may also have facilitatory roles in the action of other hormones (Figure 67).

THE ROLE OF THE THYROID HORMONES

The results of all of the experiments showed that differences in milk production were not generally related to differences in either

thyroxine (T4) or tri-iodothyronine (T3) concentration. There was, however, a tendency for T4 levels to increase particularly during early lactation in all studies, although a similar result was not observed for T3 levels.

The significance of lowered T4 levels during early lactation observed in this and earlier studies is unknown and certainly merits further study in view of the role of thyroid hormones in the control of metabolic rate (Figure 67).

The divergence of thyroid hormone levels following peak production in the dietary protein comparison and at the end of lactation in the breed comparison suggests that there may be some modification of thyroid hormone activity between treatment groups during lactation, although this difference was not always related to any divergence in milk production.

T3 is more biologically active than T4 and most of T3 is derived from T4 (Bernal and Refetoff, 1977). Therefore changes in T4 concentration may reflect changes in the rate of conversion to T3, as well as changes in T4 secretion rate and metabolic clearance rate. Thus further understanding of the significance of the observed changes in thyroid hormone concentrations will require an assessment of entry and exit rates to and from the T4 and T3 pools as well as further investigation of the biological consequences of different circulating concentrations.

GENERAL CONCLUSIONS

This work provides a basis for further studies of the mechanisms controlling milk production and serves to highlight the complex nature of the control system. Although this work confirms the importance of GH for milk production, the results suggest that levels of GH alone cannot explain all the recorded effects on circulating nutrient levels and pattern

of milk production. In future work the inter-relationships between this and other hormones, particularly insulin, should be considered. In addition to the evaluation of circulating hormone levels, studies of changes in hormone receptor numbers and affinity must be explored together with contemporary changes in enzyme and endocrine activity at the cellular level.

All these factors are important in relation to manipulation of milk production. This has already been achieved by exogenous administration of GH and T4 or thyro-active hormones. At present, GH is the most promising manipulative hormone, due to the fact that unlike T4 or the thyro-active compounds GH stimulates milk production apparently without reducing the efficiency of milk production. However, the predictability of the response is by no means certain (Bauman and McCutcheon, 1986); more precise manipulation of milk production will require a more complete understanding of the consequences of changing concentrations of one of many interacting hormones, in terms of nutrient metabolism.

Increasing milk production in the ewe is arguably appropriate in view of the recent development of techniques which have successfully improved ewe fecundity (Scaramuzzi and Hoskinson, 1984). In particular, increases may be of most value during the declining phase of lactation when increases in herbage intake do not fully compensate the fall in milk production and increased maintenance requirements of the growing lamb (Doney et al., 1983). Thus, for early lamb production, improvement in milk yield may be of considerable value. However, it is likely that the achievement of a high degree of control of level of milk production will remain difficult due to the complex and multi-factorial nature of the control system.

REFERENCES

AGRICULTURAL RESEARCH COUNCIL, (1980). The Nutrient Requirements of Ruminant Livestock. Commonwealth Agricultural Bureaux, Slough.

AGRICULTURAL RESEARCH COUNCIL., (1984). The Nutrient Requirements of Ruminant Livestock. Supplement No. 1. Commonwealth Agricultural Bureaux, Slough.

AKERS, R.M., HEALD, C.W., BIBB, T.L. and McGILLIARD, M.L. (1977). Effect of prepartum milk removal on quantitative morphology of bovine lactogensis. Journal of Dairy Science 60, 1273-1282.

AL-ANSARI, A.A.K., PERRY, L.A., SMITH, D.S. and LANDON, J. (1982). Salivary cortisol (hydrocortisone) determination: adaptation of a commercial serum cortisol kit. Annals of Clinical Biochemistry 19, 163-166.

ALEXANDER, G. (1974). Birth weight of lambs: influences and consequences. In: Size at Birth (Eds. K. Elliot and J. Knight) CIBA Foundation Symposium 27 (new series). Elsevier, Amsterdam, 215-239.

ALEXANDER, G. and DAVIES, H.L. (1959). Relationship of milk production to numbers of lambs born or suckled. Australian Journal of Agricultural Research 10, 720-724.

ANDERSON, R.R. (1975). Mammary gland growth in sheep. Journal of Animal Science 41, 118-123.

ANNISON, E.F. (1960). Plasma non-esterified fatty acids in sheep. Australian Journal of Agricultural Research 11, 58-64.

ANNISON, E.F., LINZELL, J.L., FAZAKERLEY, S. and NICHOLS, B.W. (1967). The oxidation and utilisation of palmitate, stearate, oleate and acetate by the mammary gland of the fed goat in relation to their overall metabolism and the role of plasma phospholipids and neutral lipids in milk fat synthesis. Biochemical Journal 102, 637-647.

BAIRD, G.D. (1981). Metabolic modes indicative of carbohydrate status in the dairy cow. Federation Proceedings 40, 2530-2535.

BALDWIN, B.L. and LOUIS, S. (1975). Hormonal actions on mammary metabolism. Journal of Dairy Science 58, 1033-1041.

BARNICOAT, C.R., LOGAN, A.G. and GRANT, A.I. (1949). Milk-secretion studies with New Zealand Romney ewes. Journal of Agricultural Science, Cambridge 39, 44-55.

BARTLETT, S., BURT, A.W.A., FOLLEY, S.J. and ROWLAND, S.J. (1954). Relative galactopoietic effects of 3:5:3'-Triiodo-L-thyronine and L-Thyroxine in lactating cows. Journal of Endocrinology 10, 193-201.

BASS, E., SHANI, J., GIVANT, Y., YAGIL, R and SULMAN, F.G. (1974). The effect of psychopharmacologic prolactin releasers on lactation in sheep. Archives Internationales de Pharamacodynamie et de Therapie 211, 188-192.

BASSETT, J.M. (1974a). Early changes in plasma insulin and growth hormone levels after feeding in lambs and adult sheep. Australian Journal of Biological Sciences 27, 157-166.

BASSETT, J.M. (1974b). Diurnal patterns of plasma insulin, growth hormone, corticosteriod and metabolite concentrations in fed and fasted sheep. Australian Journal of Biological Sciences 27, 167-181.

BASSETT, J.M. (1975). Dietary and gastro-intestinal control of hormones regulating carbohydrate metabolism in ruminants. In: Digestion and Metabolism in the Ruminant (Eds. I.W. McDonald and A.C.I. Warner). Proceedings of the IV International Symposium on Ruminant Physiology. University of New England Publishing Unit, Sydney, 383-398.

BASSETT, J.M. (1978). Endocrine factors in the control of nutrient utilisation: ruminants. Proceedings of the Nutrition Society 37, 273-280.

BASSETT, J.M. and HINKS, N.T. (1969). Micro-determination of corticosteriods in ovine peripheral plasma : effects of venipuncture, corticotrophin, insulin and glucose. Journal of Endocrinology 44, 387-403.

BASSETT, J.M., OXBORROW, T.J., SMITH, I.D. and THORNBURN, G.D. (1969). The concentration of progesterone in the peripheral plasma of the pregnant ewe. Journal of Endocrinology 45, 449-457.

BASSETT, J.M. and WALLACE A.L.C. (1966). Short-term effects of ovine growth hormone on plasma glucose, free fatty acids and ketones in sheep. Metabolism 15, 933-944.

BASSETT, J.M. and WALLACE, A.L.C. (1967). Influence of cortisol on plasma insulin in the sheep. Diabetes 16, 566-571.

BASSETT, J.M., WESTON, R.H. and HOGAN, J.P. (1971). Dietary regulation of plasma insulin and growth hormone concentrations in sheep. Australian Journal of Biological Sciences 24, 321-330.

BAUMAN, D.E. (1976). Intermediary metabolism of adipose tissue. Federation Proceedings 35, 2308-2313.

BAUMAN, D.E., AKERS, R.M., CHAPIN, L.T., TUCKER, H.A. and CONVEY, E.M. (1979). Effect of level of intake on serum concentrations of prolactin and growth hormone in lactating cows. Journal of Dairy Science 62 (Supplement 1), 114 (Abstract).

BAUMAN, D.E. and CURRIE, W.B. (1980). Partitioning of nutrients during pregnancy and lactation: A review of mechanisms involving homeostasis and homeorhesis. Journal of Dairy Science 63, 1514-1529.

BAUMAN, D.E. and ELLIOT, J.M. (1983). Control of nutrient partitioning in lactating ruminants. In: Biochemistry of Lactation (Ed. T.B. Mepham) Elsevier, New York, 437-468.

BAUMAN D.E. and McCUTCHEON, S.N. (1986). The effects of growth hormone and prolactin on metabolism. In: Control of Digestion and Metabolism in Ruminants (Eds. L.P. Milligan, W.L. Grovum and A. Dobson). Prentice-Hall, Englewood Cliffs, 436-455.

BERNAL, J. and REFETOFF, S. (1977). The action of thyroid hormone. Clinical Endocrinology 6, 227-249.

BINES, J.A. and HART, I.C. (1977). Milk or Meat. In: The Use of Blood Metabolites in Animal Production (Eds. D. Lister and N.G. Gregory) B.S.A.P. Occasional Publication No. 1., Milton Keynes, 87-98.

BINES, J.A. and HART, I.C. (1978). Hormonal regulation of the partition of energy between milk and body tissue in adult cattle. Proceedings of the Nutrition Society 37, 281-287.

BINES, J.A. and HART, I.C. (1982). Metabolic limits to milk production, especialy roles of growth hormone and insulin. Journal of Dairy Science 65, 1375-1389.

BINES, J.A., HART, I.C. and MORANT, S.V. (1983). Endocrine control of energy metabolism in the cow: diurnal variations in the concentrations of hormones and metabolites in the blood plasma of beef and dairy cows. Hormone and Metabolic Research 15, 330-334.

BLAXTER, K.L., REINEKE, E.P., CRAMPTON, E.W. and PETERSEN, W.E. (1949). The role of thyroidal materials and of synthetic goitrogens in animal production and an apprasial of their practical use. Journal of Animal Science 8, 307-352.

BLOM, A.K., HOVE, K. and NEDKVITNE, J.J. (1976). Plasma insulin and growth hormone concentrations in pregnant sheep. II: Post-absorptive levels in mid- and late pregnancy. Acta Endocrinologica 82, 553-560.

BOLTON, S.C., WEEKES, T.E.C., GODDEN, P.M.M. and ARMSTRONG, D.G. (1983). Glucagon-induced lipolysis and plasma Mg levels in normal and hyperthyroid sheep. Proceeding of the Nutrition Society 42, 48A (Abstract).

BOSC, M.J. (1972). The induction and synchronisation of lambing with the aid of dexamethasone. Journal of Reproduction and Fertility 28, 347-357.

BOWDEN, D.M. (1971). Non-esterified fatty acids and ketone bodies in blood as indicators of nutritional status in ruminants: a review. Canadian Journal of Animal Science 51, 1-13.

BRITISH STANDARD 696, Part 2. (1969). Gerber method for the determination of fat in milk and milk products. British Standards Institute, London.

BROCKMAN, R.P. (1978). Roles of glucagon and insulin in the regulation of metabolism in ruminants: a review. Canadian Veterinary Journal 19, 55-62.

BURRIS, M.J. and BAUGUS, C.A. (1955). Milk consumption and growth of suckling lambs. Journal of Animal Science 14, 186-191.

BUTLER, W.R., FULLENCAMP, S.M., CAPIELLO, L.A. and HANDWERGER, S. (1981). The relationship between breed and litter size in sheep and maternal serum concentrations of placental lactogen, estradiol and progesterone. Journal of Animal Science 53, 1077-1081.

BUTLER-HOGG, B.W. and WHELEHAN, O,P. (1986). Carcass quality of dairy sheep. Animal Production 42, 461 (Abstract).

CALDERON CORTES, J.F., ROBINSON, J.J., McHATTIE, I. and FRASER, C. (1977). The sensitivity of ewe milk yield to changes in dietary crude protein concentration. Animal Production 24, 135 (Abstract).

CHESWORTH, J.M. and EASDON, M.P. (1983). Effect of diet and season on steriod hormones in the ruminant. Journal of Steriod Biochemistry 19, 715-723.

COGGINS, C.R.E. and FIELD, A.C. (1978). Changes in plasma concentrations of glucose, free fatty acids, ketone bodies, thyroxine and insulin of lactating beef cows in relation to time of feeding and energy status. In: The Use of Blood Metabolites in Animal Production (Eds. D. Lister and N.G. Gregory) B.S.A.P. Occasional Publication No. 1., Milton Keynes, 41-48.

COLLIER, R.J., McNAMARA, J.P., WALLACE, C.R. and DEHOFF, M.H. (1984). A review of endocrine regulation of metabolism during lactation. Journal of Animal Science 59, 498-510.

CONVEY, E.M. (1974). Serum hormone concentrations in ruminants during mammary growth, lactogenesis, and lactation : a review. Journal of Dairy Science 57, 905-917.

CORBETT, J.L. (1968). Variation in yield and composition of milk of grazing Merino ewes. Australian Journal of Agricultural Research 19, 283-294.

COWAN, R.T., ROBINSON, J.J., GREENHALGH, J.F.D. and McHATTIE, I. (1979). Body composition changes in lactating ewes estimated by serial slaughter and deuterium dilution. Animal Production 29, 81-90.

COWAN, R.T., ROBINSON, J.J. and McDONALD, I. (1982). A note on the effects of body fatness and level of food intake on the rate of fat loss in lactating ewes. Animal Production 34, 355-357.

COWAN, R.T., ROBINSON, J.J., McDONALD, I. and SMART, R. (1980). Effects of body fatness at lambing and diet in lactation on body tissue loss, feed intake and milk yield of ewes in early lactation. Journal of Agricultural Science, Cambridge 95, 497-514.

COWAN, R.T., ROBINSON, J.J., McHATTIE, I. and PENNIE, K. (1981). Effects of protein concentration in the diet on milk yield, change in body composition and the efficiency of utilisation of body tissue for milk production in ewes. Animal Production 33, 111-120.

COWIE, A.T., FORSYTH, I.A. and HART, I.C. (1980). Hormonal Control of Lactation. Monographs on Endocrinology, Springer-Verlag, New York.

DAVIS, H.L. (1963). The milk production of Merino ewes at pasture. Australian Journal of Agricultural Research 14, 824-838.

DAVIS, S.L. (1972). Plasma levels of prolactin, growth hormone and insulin in sheep following the infusion of arginine, leucine and phenylalanine. Endocrinology 91 549-555.

DELOUIS C. and DENAMUR D. (1967). Induction experimentale de la secretion lactee pendant la gestation de la Brebis. Comptes Rendus Academic des Sciences de l'Academie des Sciences D 264, 2493-2496.

DELOUIS, C., DJIANE, J., HOUDEBINE, L.M. and TERQUI, M. (1980). Relation between hormones and mammary gland function. Journal of Dairy Science 63, 1492-1513.

DENAMUR, R. (1965). Les acides nucleiques et les nucleotides libres de la glande mammaire pendant la lactogenese et la galactopoiese. Exerpta Medica International Congress Series 83 Part I, 432-462.

DENAMUR, R. (1971). Reviews of the progress of dairy science. Section A Physiology. Hormonal control of lactogenesis. Journal of Dairy Research 38 237-264.

DENAMUR, R. and MARTINET, J. (1961). Effets de l'hypophysectomie et de la section de la tige pituitaire sur la gestation de la brebis. Annales D'Endocrinologie 22, 755-759.

DENAMUR, R. and MARTINET, J. (1970). Nature des hormones ante-hypophysaires stimulantes de la secretion lactee chez la brebis normale. Archives de Pharmacodynamie et de Therapie 186, 185-187.

DJIANE, J. and KANN, G. (1975). Mise en evidence de l'activite lactogene et mesure dans le serum de l'activite prolactinique du placenta chez la brebis au cours de la gestation. Comptes Rendus Academie des Sciences, de l'Academie des Sciences D. 280, 2785-2788.

DONEY, J.M. and MUNRO, J. (1962). The effect of suckling, management and season on sheep milk production as estimated by lamb growth. Animal Production 4, 215-220.

DONEY, J.M., PEART, J.N. and SMITH, W.F. (1981). The effect of interaction of ewe and lamb genotype on milk production of ewes and on growth of lambs to weaning. Animal Production, 33, 137-142.

DONEY, J.M., PEART, J.N., SMITH, W.F. and LOUDA, F. (1979). A consideration of the techniques for estimation of milk yield by suckled sheep and a comparison of estimates obtained by two methods in relation to the effect of breed, level of production and stage of lactation. Journal of Agricultural Science, Cambridge 92, 123-132.

DONEY, J.M., PEART, J.N., SMITH, W.F. and SIM, D.A. (1983). Lactation performance, herbage intake and lamb growth of Scottish Blackface and East Friesland X Scottish Blackface ewes grazing hill or improved pasture. Animal Production 37, 283-292.

DRIVER, P.M. and FORBES, J.M. (1981). Episodic growth hormone secretion in sheep in relation to time of feeding, spontaneous meals and short term fasting. Journal of Physiology 317, 413-424.

FAICHNEY, G.J. and WESTON, R.H. (1971). Digestion by ruminant lambs of a diet containing formaldehyde-treated casein. Australian Journal of Agricultural Research 22 461-468.

FAILING, J.F., BUCKLEY, M.W. and ZAK, B. (1960). Automatic determination of serum proteins. Americal Journal of Clinical Pathology 33, 83-88.

FELL, L.R., BECK, C., BROWN, J.M., KATT, K.J., CUMMING, I.A. and GODING, J.R. (1972). Solid-phase radioimmunoassay of ovine prolactin in antibody-coated tubes. Prolactin secretion during estradiol treatment, at parturition and during milking. Endocrinology 91, 1329-1336.

FELL, B.F., CAMPBELL, M., MACKIE, W.S. and WEEKES, T.E.C. (1972). Changes associated with pregnancy and lactation in some extra-reproductive organs of the ewe. Journal of Agricultural Science, Cambridge 79, 397-407.

FLAMANT, J.C. and CASU, S. (1978). Breed differences in milk production potential and genetic improvement of milk production. In: Milk Production in the Ewe (Eds. J.G. Boyazaglu and T.T.Treacher) E.A.A.P. Publication No. 23, 1-20.

FOOT, J.Z., CUMMINS, L.J., SPIKER, S.A. and FLINN, P.C. (1984). Concentration of B-hydroxybutyrate in plasma of ewes in late pregnancy and early lactation and survival and growth of lambs. In: Reproduction in Sheep (Eds D.R. Lindsay and D.T. Pearce) Australian Academy of Science with the Australian Wool Corporation, Canberra, 187-190.

FOOT, J.Z., SKEDD, E. and McFARLENE D.N. (1979). Body composition in lactating sheep and its indirect measurement in the live animal using tritiated water. Journal of Agricultural Science, Cambridge 92, 69-81.

FOOT, J.Z. and TISSIER, M. (1978). Voluntary intake of feed by lactating ewes. In: Milk Production in the Ewe (Eds. J.G. Boyazoglu and T.T. Treacher) E.A.A.P. Publication No.23, 66-75.

FORBES, J.M. (1982). Effects of lighting pattern on growth, lactation and food intake of sheep, cattle and deer. Livestock Production Science 9, 361-374.

FORBES, J.M., DRIVER, P.M., BROWN, B., SCANES, C.G. and HART, I.C. (1979). The effect of daylength on the growth of lambs. 2. Blood concentrations of growth hormone, prolactin, insulin and thyroxine, and the effect of feeding. Animal Production 29, 43-51.

FORBES, J.M., DRIVER, P.M., EL SHAHAT, A.A., BOAZ, T.G. and SCANES, C.G. (1975). The effect of daylength and level of feeding on serum prolactin in growing lambs. Journal of Endocrinology 64, 549-554.

FORSYTH I.A. (1971). Organ culture techniques and the study of hormone effects on the mammary gland. Journal of Dairy Research 38, 419-444.

FORSYTH I.A. (1983). The endocrinology of lactation. In: Biochemistry of Lactation (Ed T.B. Mepham) Elsevier, New York, 309-349.

FULKERSON, W.J. (1979). Hormonal Control of Lactation. Volume 1. Eden Press, Quebec.

FULKERSON, W.J. and McDOWELL, G.H. (1974). Artificial induction of lactation in ewes. Journal of Endocrinology 63, 167-173.

FULKERSON, W.J., McDOWELL, G.H. and FELL, L.R. (1975). Artificial induction of lactation in ewes: the role of prolactin. Australian Journal of Biological Sciences 28, 525-530.

GARDNER, R.W. and HOGUE, D.E. (1964). Effects of energy intake and number of lambs suckled on milk yield, milk composition and energetic efficiency of lactating ewes. Journal of Animal Science 23, 935-942.

GARDNER, R.W. and HOGUE, D.E. (1966). Milk production, milk composition and energetic efficiency of Hampshire and Corriedale ewes fed to maintain body weight. Journal of Animal Science 25, 789-795.

GIBB, M.J. and TREACHER, T.T. (1982). The effect of body condition and nutrition during late pregnancy on the performance of grazing ewes during lactation. Animal Production 34, 123-129.

GIBB, M.J., TREACHER, T.T. and SHANMUGALINGAM, V.S. (1981). Herbage intake and performance of grazing ewes and of their lambs when weaned at 6, 8, 10 or 14 weeks of age. Animal Production 33, 223-232.

GONZALEZ, J.S., ROBINSON, J.J., McHATTIE, I. and FRASER, C. (1982). The effect in ewes of source and level of dietary protein on milk yield, and the relationship between the intestinal supply of non-ammonia N and the production of milk protein. Animal Production 34, 31-40.

GOW, C.B., McDOWELL, G.H. and ANNISON, E.F. (1981). Control of gluconeogenesis in the lactating sheep. Australian Journal of Biological Sciences 34, 469-478.

HADJIPIERIS, G. and HOLMES, W. (1966). Studies of feed intake and feed utilisation by sheep. I. The voluntary feed intake of dry, pregnant and lactating ewes. Journal of Agricultural Science, Cambridge 66, 217-223.

HANDWERGER, S., CRENSHAW, C., MAURER, W.F., BARRETT, J., HURLEY, T.W., GOLANDER, A. and FELLOWS, R.E. (1977). Studies on ovine placental lactogen secretion by homologuous radioimmunoassay. Journal of Endocrinology 72, 27-34.

HART, I.C. (1973). Effect of 2-bromo-d-ergocryptine on milk yield and the level of prolactin and growth hormone in the blood of the goat at milking. Journal of Endocrinology 57, 179-180.

HART, I.C. (1981). Secretion and action of growth hormone. In: Hormones and Metabolism in Ruminants. ARC Workshop Proceedings, 47-64.

HART, I.C. (1983). Endocrine control of nutrient partition in lactating ruminants. Proceedings of the Nutrition Society 42, 181-194.

HART, I.C., BINES, J.A., BALCH, C.C. and COWIE, A.T. (1975). Hormone and metabolite differences between lactating beef and dairy cattle. Life Sciences 16, 1285-1292.

HART, I.C., BINES, J.A. and MORANT, S.V. (1979). Endocrine control of energy metabolism in the cow: Correlations of hormones and metabolites in high- and low-yielding cows, for stage of lactation. Journal of Dairy Science 62, 270-277.

HART, I.C., BINES, J.A., MORANT, S.V. and RIDLEY, J.L. (1978). Endocrine control of energy metabolism in the cow: Comparison of the levels of hormones (prolactin, growth hormone, insulin and thyroxine) and metabolites in the plasma of high- and low- yielding cattle at various stages of lactation. Journal of Endocrinology 77, 333-345.

HART, I.C., CHADWICK, P.M.E., JAMES, S. and SIMMONDS, A.D. (1985). Effects of intravenous bovine growth hormone or human pancreatic growth hormone-releasing factor on milk production and plasma hormones and metabolites in sheep. Journal of Endocrinology 105, 189-196.

HART, I.C., FLUX, D.S., ANDREWS, P. and McNEILLY, A.S. (1975). Radioimmunoassay for ovine and caprine growth hormone: its application to the measurement of basal circulating levels of growth hormone in the goat. Hormone and Metabolic Research 7, 35-40.

HART, I.C. and LINZELL, J.L. (1977). An analysis of specific stimuli causing the release of prolactin and growth hormone at milking in the goat. Journal of Endocrinology 72, 163-171.

HARTMANN, P.E., TREVETHAN, P. and SHELTON, J.N. (1973). Progesterone and oestrogen and the initiation of lactation in ewes. Journal of Endocrinology 59, 249-259.

HAYDEN, T.J., THOMAS, C.R. and FORSYTH, I.A. (1979). Effect of number of young born (litter size) on milk yield of goats: role for placental lactogen. Journal of Dairy Science 62, 53-57.

HAYDEN, T.J., THOMAS, C.R., SMITH, S.V. and FORSYTH, I.A. (1980). Placental lactogen in the goat in relation to stage of gestation, number of festuses, metabolites, progesterone and time of day. Journal of Endocrinology 86, 279-290.

HEAD, H.H., DELOUIS, C., TERQUI, M., KANN, G. and DJIANE, J. (1980). Effects of various hormone treatments on induction of lactation in the ewe. Journal of Animal Science 50, 706-712.

HERTELENDY, F. and KIPNIS, D.M. (1973). Studies on growth hormone secretion: V. Influence of plasma free fatty acid levels. Endocrinology 92, 402-410.

HOOLEY, R.D., CAMPBELL, J.J. and FINDLAY, J.K. (1978). The importance of prolactin for lactation in the ewe. Journal of Endocrinology 79, 301-310.

HOUDEBINE, L., DJIANE, J. DUSANTER-FOURT, I., MARTEL, P., KELLY, P.A., DEVINOY, E. and SERVELY, J. (1985). Hormonal action controlling mammary activity. Journal of Dairy Science 68, 489-500.

HOVE, K. and BLOM, A.K. (1973). Plasma insulin and growth hormone in dairy cows: Diurnal variation and relation to food intake and plasma sugar and acetoacetate levels. Acta Endocrinologica 73, 289-303.

JAGUSCH, K.T., JAY, N.P. and CLARK, V.R. (1972). Nutrition of the ewe in early lactation. II. Milk yield. New Zealand Journal of Agricultural Research 15, 209-213.

JENNY, B.F., POLAN, C.E. and THYE, F.W. (1974). Effects of high grain feeding and stage of lactation on serum insulin, glucose and milk fat percentage in lactating cows. Journal of Nutrition 104, 379-385.

JEULIN-BAILLY, C., DELOUIS, C. and DENAMUR, R. (1973). Influence de l'oestradiol 17B et de la progesterone au cours de l'induction par la prolactine et l'hormone de croissance de la differentiation lobuloalveolaire du tissu mammaire de brebis gestante: etude en culture organotypique. Comptes Rendus Academie des Sciences, de l'Academie des Scienes D 277, 2525-2528.

JOHNSON, H.D. and VANJONACK, W.D. (1976). Effects of environmental and other stressors on blood hormone patterns in lactating animals. Journal of Dairy Science 59, 1603-1617.

JORDAN, R.M. and SHAFFHAUSEN, D.D. (1954). Effect of somatotrophin on milk yield in ewes. Journal of Animal Science 13, 706-710.

KANN, G. (1976). Inhibition of prolactin secretion in the ewe by 2-Br-ergocryptin during pregnancy or early lactation; Effect on milk yield. Proceedings of the Vth International Congress of Endocrinology, Hamburg 251 (Abstract).

KELLY, P.A., ROBERTSON, H.A. and FRIESEN, H.G. (1974). Temporal pattern of placental lactogen and progesterone secretion in sheep. Nature 248, 435-437.

KNIGHT, C.H. and PEAKER, M. (1982a). Effects of fasting during mid pregnancy or early lactation on mammary development and milk yield in mice. Journal of Dairy Research 49, 567-575.

KNIGHT, C.H. and PEAKER, M. (1982b). Development of the mammary gland. Journal of Reproduction and Fertility 65, 521-536.

KOPROWSKI, J.A. and TUCKER, H.A. (1973a). Serum prolactin during various physiological states and its relationship to milk production in the bovine. Endocrinology 92, 1480-1487.

KOPROWSKI, J.A. and TUCKER, H.A. (1973b). Bovine serum growth hormone, corticoids and insulin during lactation. Endocrinology 93, 645-651.

KOVNEREV, I.P. (1974). Biologicheskie rezervy romanovskogo ovtsevodstva [Biological reserves of Romanov sheep]. Ovtesevodstvo 11, 29-30 (Animal Breeding Abstracts 43, 2944).

KRONFIELD, D.S. (1976). The potential importance of glucogenic, lipogenic and aminogenic nutrients in regard to the health and productivity of dairy cows. Advances in Animal Physiology and Animal Nutrition 1, 5-26.

KRONFIELD, D.S., MAYER, G.P., ROBERTSON, J.M., RAGGI, F. (1963). Depression of milk secretion during insulin administration. Journal of Dairy Science 46, 559-564.

KUHN, N.J. (1983). The biochemistry of lactogenesis. In: Biochemistry of Lactation (Ed. T.B. Mepham) Elsevier, New York, 351-379.

LAMMING, G.E., MOSELEY, S.R. and McNEILLY, J.R. (1972). Prolactin release in the ewe at parturition and first suckling. Journal of Endocrinology 55, XXVII (Abstract).

LAMMING, G.E., MOSELEY, S.R. and McNEILLY, J.R. (1974). Prolactin release in the sheep. Journal of Reproduction and Fertility. 40 151-168.

LANGLANDS, J.P. (1972). Growth and herbage consumption of grazing Merino and Border Leicester lambs reared by their mothers or fostered by ewes of the other breed. Animal Production 14, 317-322.

LINDSAY, D.B. (1978). The effect of feeding pattern and sampling procedure on blood parameters. In: The Use of Blood Metabolites in Animal production (Eds. D. Lister and N.G. Gregory) B.S.A.P. Occasional Publication No. 1. Milton Keynes, 99-120.

LOERCH, S.C., McCLURE, K.E. and PARKER, C.F. (1985). Effects of number of lambs suckled and supplemental protein source on lactating ewe performance. Journal of Animal Science 60, 6-13.

LOMAX, M.A., BAIRD, G.D., MALLINSON, C.B. and SYMONDS, H.W. (1979). Differences between lactating and non-lactating dairy cows in concentration and secretion rate of insulin. Biochemical Journal 180, 281-289.

LOUDA, F. and DONEY, J.M. (1976). Persistency of lactation in the Improved Valachian breed of sheep. Journal of Agricultural Science, Cambridge 87, 455-457.

LOUW, B.P., LISHMAN, A.W. and BOTHA, W.A. (1974). Stress-induced release of prolactin in cycling and anoestrous ewes, and in wethers. South African Journal of Animal Science 4, 131-135 (Animal Breeding Abstracts 43, 3472).

MAINOYA, J.R. (1978). Possible influence of prolactin on intestinal hypertrophy in pregnant and lactating rats. Experientia 34, 1230-1231.

MANNS, J.G. and BODA, J.M. (1965). Effects of ovine growth hormone and prolactin on blood glucose, serum insulin, plasma non-esterified fatty acids and amino nitrogen in sheep. Endocrinology 76, 1109-1114.

MARTAL, J. (1975). Effects of hand-milking on growth hormone levels in the ewe and goat. Comptes Rendus des Seances, de l'Academie des Sciences D 280, 197-200.

MARTAL, J. and DJIANE, J. (1977a). The production of chorionic somatomammotrophin in sheep. Journal of Reproduction and Fertility 49, 285-289.

MARTAL, J. and DJIANE, J. (1977b). Mammotrophic and growth promoting activities of a placental hormone in sheep. Journal of Steriod Biochemistry 8, 415-417.

MAXWELL, T.J., DONEY, J.M., MILNE, J.A., PEART, J.N., RUSSEL, A.J.F., SIBBALD, A.R. and MACDONALD, D. (1979). The effect of rearing type and prepartum nutrition on the intake and performance of lactating Greyface ewes at pasture. Journal of Agricultural Science, Cambridge 92, 165-174.

MAULE WALKER, F.M. (1984). Exocrine and endocrine secretions of the mammary gland: local control in ruminants. In: Physiological Strategies in Lactation (Eds. M. Peaker, R.G. Vernon and C.H. Knight) Symposia of the Zoological Society of London No. 51. Academic Press, London, 171-191.

McATEE, J.W. and TRENKLE, A. (1971). Effects of feeding, fasting, glucose or arginine on plasma prolactin levels in the bovine. Endocrinology 89, 730-734.

McCANCE I. (1959). The determination of milk yield in the Merino ewe. Australian Journal of Agricultural Research 10 839-853.

McCANCE, I. and ALEXANDER, G. (1959). The onset of lactation in the Merino ewe and its modification by nutritional factors. Australian Journal of Agricultural Reserach 10, 699-719.

McDOWELL, G.H. (1983). Hormonal control of glucose homeostatsis in ruminants. Proceedings of the Nutrition Society 42, 149-167.

McDOWELL, G.H. and HART, I.C. (1984). Responses to infusion of growth hormone into the mammary arteries of lactating sheep. Canadian Journal of Animal Science 64 (Supplement), 306-307.

McDOWELL, G.H. and HART, I.C. (1983). Galactopoietic effects of bovine growth hormone in lactating sheep and goats. Proceedings of the Fifteenth Annual Conference of the Australian Society for Reproductive Biology, 96.

McINTYRE, K.H. (1970). The effects of increased nitrogen intakes on plasma urea nitrogen and rumen ammonia levels in sheep. Australian Journal of Agricultural Research 21, 501-507.

McNATTY, K.P., CASHMORE, M., YOUNG, A. (1972). Diurnal variation in plasma cortisol levels in sheep. Journal of Endocrinology 54, 361-362.

McNEILLY, J.R. (1971). A solid phase radioimmunoassay for ovine prolactin. Journal of Endocrinology 49, 141-149.

McNEILLY, A.S. and ANDREWS, P. (1974). Purification and characterization of caprine prolactin. Journal of Endocrinology 60, 359-367.

McNEILLY, J.R., MOSELEY, S.R. and LAMMING, G.E. (1972). Observations on the pattern of prolactin release during suckling in the ewe. Journal of Reproduction and Fertility 31, 487-488.

MEAT AND LIVESTOCK COMMISSION (1981). Feeding the Ewe. Second Edition Sheep Improvement Services. M.L.C., Milton Keynes, England

MEITES, J. (1961). Farm animals: hormonal induction of lactation and galactopoiesis. In: Milk : The mammary gland and its secretion (Eds. S.K. Kon and A.T. Cowie), Vol 1, Acaemic Press, London, 321-367.

MELLOR, D.J. (1983). Nutritional and placental determinants of foetal growth rate in sheep and consequences for the newborn lamb. British Veterinary Journal 139, 307-324.

MELLOR, D.J. and MURRAY, L. (1985). Effects of maternal nutrition on udder development during late pregnancy and on colostrum production in Scottish Blackface ewes with twin lambs. Research in Veterinary Science 39, 230-234.

MEPHAM, T.B. (1982). Amino acid utilisation by lactating mammary gland. Journal of Dairy Science 65, 287-298.

MEPHAM, T.B., LAWRENCE, S.E., PETERS, A.R. and HART, I.C. (1984). Effects of exogenous growth hormone on mammary function in lactating goats. Hormone and Metabolic Research 16, 248-253.

MILLS, S.E. and JENNY, B.F. (1979). Effects of high concentrate feeding and fasting on plasma glucocorticoids in dairy heifers. Journal of Animal Science 48, 961-965.

MOORE, R.W. (1966). Genetic factors affecting the milk intake of lambs. Australian Journal of Agricultural Research. 17, 191-199.

MORAG, M., SHANI, J., SULMAN, F.G. and YAGIL, R. (1971). The effect of perphenazine on mammary development and milk yield in the dairy ewe. Journal of Endocrinology 49, 351-352.

OLDHAM, J.D. (1984). Protein-energy interrelationships in dairy cows. Journal of Dairy Science 67, 1090-1114.

OWEN, J.B.. (1957). A study of the lactation and growth of hill sheep in their native environment and under lowland conditions. Journal of Agricultural Science, Cambridge 48, 387-411.

PATERSON, D.S.P. (1963). Some observations on the estimation of non-esterified fatty acid concentrations in cow and sheep plasma. Research in Veterinary Science 4, 230-237.

PEART, J.N. (1967). The effect of difference levels of nutrition during late pregnancy on the subsequent milk production of Blackface ewes and on the growth of their lambs. Journal of Agricultural Science, Cambridge 68, 365-371.

PEART, J.N. (1968). Lactation studies with Blackface ewes and their lambs. Journal of Agricultural Science, Cambridge 70, 87-94.

PEART, J.N. (1970a). Factors influencing lactation of hill ewe. Hill Farming Research Organisation 5th Report (1967-1970), 55-69.

PEART, J.N. (1970b). The influence of live weight and body condition on the subsequent milk production of Blackface ewes following a period of undernourishment in early lactation. Journal of Agricultural Science, Cambridge 75, 459-469.

PEART, J.N. (1973). Milk production of Westphalian Milch sheep. HFRO Annual Report, 6.

PEART, J.N., DONEY, J.M. and MACDONALD, A.J. (1975). The influence of lamb genotype on the milk production of Blackface ewes. Journal of Agricultural Science, Cambridge 84, 313-316.

PEART, J.N., DONEY, J.M. and SMITH, W.F. (1979). Lactation pattern in Scottish Blackface and East Friesland X Scottish Blackface crossbred ewes. Journal of Agricultural Science, Cambridge 92, 133-138.

PEART, J.N., EDWARDS, R.A. and DONALDSON, E. (1972). The yield and composition of the milk of Finnish Landrace X Blackface ewes. I. Ewes and lambs maintained indoors. Journal of Agricultural Science, Cambridge 79, 303-313.

PEART, J.N., EDWARDS, R.A. and DONALDSON, E. (1975). The yield and composition on the milk and Finnish Landrace X Blackface ewes. II. Ewes and lambs grazed on pasture. Journal of Agricultural Science, Cambridge 85, 315-324.

PEART, J.N. (1982). Lactation of suckling ewes and does. In: Sheep and Goat Production (Ed. I.E. Coop). World Animal Sciences Elsevier Oxford, 119-134.

PEEL, C.J., BAUMAN, D.E., GOREWIT, R.C. and SNIFFEN, C.J. (1981). Effect of exogenous growth hormone on lactational performance in high yielding dairy cows. Journal of Nutrition 111, 1662-1671.

PELLETIER, J. (1973). Evidence for photoperiodic control of prolactin release in rams. Journal of Reproduction and Fertility 35, 143-147.

PIPES, G.W., BAUMAN, T.R., BROOKS, J.R., COMFORT, J.E. and TURNER, C.W. (1963). Effect of season, sex and breed on the thyroxine secretion rate of beef cattle and a comparison with dairy cattle. Journal of Animal Science 22, 476-480.

RATTRAY, P.V., GARRETT, W.N., EAST, N.E. and HINMAN, N. (1974). Growth, development and composition of the ovine conceptus and mammary gland during pregnancy. Journal of Animal Science 38, 613-626.

RAVAULT, J.P. (1976). Prolactin in the ram: Seasonal variations in the concentration of blood plasma from birth until three years old. Acta Endocrinologica 83, 720-725.

REID, R.L. and HINKS, N.T. (1962a). Studies of the carbohydrate metabolism of sheep. XVII. Feed requirements and voluntary feed intake in late pregnancy, with particular reference to prevention of hypoglycaemia and hyperketonaemia. Australian Journal of Agricultural Research 13, 1092-1111.

REID, R.L. and HINKS, N.T. (1962b). Studies on the carbohydrate metabolism of sheep XVIII. The metabolism of glucose, free fatty acids, ketones and amino acids in late pregnancy and lactation. Australian Journal of Agricultural Research 13, 1112-1123.

REILLY, P.E.B. and BLACK, A.L. (1973). Early effects of cortisol on glucose and alanine metabolim in adrenalectomised sheep. American Journal of Physiology 225, 689-695.

REINHARDT, V. and SCHAMS, D. (1974). Analysis of teat stimulation as a specific stimulus for prolactin in cattle. Neuroendocrinology 14, 289-296.

REINHARDT, V. and SCHAMS, D. (1975). Prolactin release in dairy heifers relative to duration of teat stimulation. Neuroendocrinology 17, 54-61.

RICHARDSON, T. (1977). A modification of the Trinder AutoAnalyzer method for glucose. Annals of Clinical Biochemistry 14, 223-226.

RICORDEAU, G. and FLAMANT, J.C. (1969). Croisements entre les races ovines Prealpes du Sud et Frisonne (Ostfriesisches Milchschaf). III Performances laitieres [Crossbreeding between Prealpes du Sud and East Friesian sheep. III. Milk yields]. Annales de Zootechnie 18, 151-168 (Animal Breeding Abstracts 38, 3703).

ROBINSON, J.J. (1973). Some aspects of ewe nutrition. Veterinary Record 92, 602-606.

ROBINSON, J.J. (1978). Response of the lactating ewe to variation in energy and protein intake. In: Milk Production in the Ewe (Eds. J.G. Boyazoglu and T.T. Treacher) E.A.A.P. Publication No. 23, 53-65.

ROBINSON, J.J. (1980). Energy requirements of ewes during late pregnancy and early lactation. Veterinary Record 106, 282-284.

ROBINSON, J.J., FRASER, C., GILL, J.C. and McHATTIE, I. (1974). The effect of dietary crude protein concentration and time of weaning on milk production and body weight change in the ewe. Animal Production 19, 331-339.

RUSSEL, A.J.F. (1978). The use of measurements of energy status in pregnant ewes. In: The use of Blood Metabolites in Animal production (Eds. D. Lister and N.G. Gregory) B.S.A.P. Occasional Publication No. 1, Milton Keynes, 31-40.

RUSSEL, A.J.F., DONEY, J.M. and GUNN, R.G. (1969). Subjective assessment of body fat in live sheep. Journal of Agricultural Science, Cambridge 72, 451-454.

RUSSEL, A.J.F., DONEY, J.M. and REID, R.L. (1967). The use of biochemical parameters in controlling nutritional state in pregnant ewes, and the effect of undernourishment during pregnancy on lamb birth-weight. Journal of Agricultural Science, Cambridge 68 351-358.

SALACINSKI, P.R.P., McLEAN, C., SYKES, J.E.C., CLEMENT-JONES, V.V. and LOWRY, P.J. (1981). Iodination of proteins, glycoproteins, and peptides using a solid-phase oxisizing agent, 1,3,4,6, Tetrachloro-3α, 6α-diphenyl Glycoluril (Iodogen). Analytical Biochemistry 117, 136-146.

SCARAMUZZI, R.J. and HOSKINSON, R.M. (1984). Active immunization against steriod hormones for increasing fecundity. In: Immunological Aspects of Reproduction in Mammals (Ed D.B. Crighton) Butterworths, London, 445-474.

SCHAMS, D. RUSSE, I. SCHALLENBERGER, E., PROKOPP, S. and CHAN, J.S.D. (1984). The role of steriod hormones, prolactin and placental lactogen on mammary gland development in ewes and heifers. Journal of Endocrinology 102, 121-130.

SEJRSEN, K., HUBER, J.T. and TUCKER, H.A. (1983). Influence of amount fed on hormone concentrations and their relationship to mammary growth in heifers. Journal of Dairy Science 66, 845-855.

SEJRSEN, K., HUBER, J.T., TUCKER, H.A. and AKERS, R.M. (1982). Influence of nutrition on mammary development in pre- and post-pubertal heifers. Journal of Dairy Science 65, 793-800.

SLEN, S.B., CLARK, R.D. and HIRONAKA, R. (1963). A comparison of milk production and its relation to lamb growth in five breeds of sheep. Canadian Journal of Animal Science 43, 16-21.

SMITH, V.G., CONVEY, E.M. and EDGERTON, L.A. (1972). Bovine serum corticoid response to milking and exteroceptive stimuli. Journal of Dairy Science 55, 1170-1173.

SPENCER, K. and PRICE, C.P. (1977). Influence of reagent quality and reaction conditions on the determination of serum albumin by the bromocresol green dye-binding method. Annals of Clinical Biochemistry 14, 105-115.

SYKES, A.R. (1978). An assessment of the value of plasma urea nitrogen and albumin concentrations as monitors of the protein status of sheep. In: The Use of Blood Metabolites in Animal Production (Eds. D. Lister and N.G. Gregory) B.S.A.P. Occasional Publication No. 1, Milton Keynes 143-154.

TAYLOR, M.J., JENKIN, G., ROBINSON, J.S., THORNBURN, G.D., FRIESEN, H. and CHAN, J.S.D. (1980). Concentrations of placental lactogen in chronically catheterized ewes and fetuses in late pregnancy. Journal of Endocrinology 85, 27-34.

THILSTED, S.H. (1985). Regulation of the partition of nutrients in the dairy cow in late pregnancy and early lactation. Zeitchrift fur Tierphysiologie Tierernatirung und Futternittelkunde 53, 10-18

THOMAS, J.W. and MOORE, L.A. (1953). Thyroprotein feeding to dairy cows during successive lactations. Journal of Dairy Science 36, 657-672.

THOMPSON, A.M. and THOMPSON, W. (1948-9). Lambing in relation to diet of the pregnant ewe. British Journal of Nutrition 2, 290-305.

THORBURN, G.D., NICOL, D.H., BASSETT, J.M., SHUTT, D.A. and COX, R.I. (1972). Parturition in the goat and sheep: changes in corticosteriods, progesterone, oestrogens and prostaglandin F. Journal of Reproduction and Fertility, Supplement 16, 61-84.

TINDAL, J.S., KNAGGS, G.S., HART, I.C. and BLAKE, A. (1978). Release of growth hormone in lactating and non-lactating goats in relation to behaviour, stages of sheep, electroencephalograms environmental stimuli and levels of prolactin, insulin, glucose and free fatty acids in the circulation. Journal of Endocrinology 76, 333-346.

TREACHER, T.T. (1970). Effects of nutrition in late pregnancy on subsequent milk production in ewes. Animal Production 12, 23-36.

TREACHER, T.T. (1971). Effects of nutrition in pregnancy and in lactation on milk yield in ewes. Animal Production 13, 493-501.

TREACHER, T.T. (1978). The effects on milk production of the number of lambs suckled and age, parity and size of ewe. In: Milk Production in the Ewe (Eds. J.G. Boyazaglu and T.T. Treacher) E.A.A.P. Publication No. 23, 31-40.

TREACHER, T.T. (1983). Nutrient requirements for lactation in the ewe. In: Sheep Production (Eds. W. Haresign) Butterworths, London, 133-153.

TRENKLE, A. (1970). Effects of short-chain fatty acids, feeding, fasting and type of diet on plasma insulin levels in sheep. Journal of Nutrition 100, 1323-1330.

TRENKLE, A. (1971). Effect of diet upon levels of plasma growth hormone in sheep. Journal of Animal Science 32, 111-114.

TRENKLE, A. (1978). Relation of hormonal variations to nutritional studies and metabolism of ruminants. Journal of Dairy Science 61, 281-293.

TRENKLE, A. (1981). Endocrine regulation of energy metabolism in ruminants. Federation Proceedings 40, 2536-2541.

TUCKER, H.A. (1981). Physiological control of mammary growth, lactogenesis, and lactation. Journal of Dairy Science 64 1403-1421.

TYRELL, H.F., BROWN, A.C.G., REYNOLDS, P.J., HAALAND, G.L., PEEL, C.J., BAUMAN, D.E. and STEINHOUR, W.D. (1982). Administration of bovine growth hormone to high yielding Holstein cows. I. Influence on in vivo energy metabolism. Journal of Dairy Science 65, Supplement, 120 (Abstract).

VANJONACK, W.J. and JOHNSON, H.D. (1975). Effects of moderate heat and milk yield on plasma thyroxine in cattle. Journal of Dairy Science 58, 507-511.

VERNON, R.G. (1980). Lipid metabolism in the adipose tissue of ruminant animals. Lipid Research 19, 23-106.

VERNON, R.G. (1982). Effects of growth hormone on fatty acid synthesis in sheep adipose tissue. International Journal of Biochemistry 14, 255-258.

VERNON, R.G., CLEGG, R.A. and FLINT, D.J. (1981). Metabolism of sheep adipose tissue during pregnancy and lactation : Adaptatin and regulation. Biochemical Journal 200, 307-314.

VERNON, R.G., CLEGG, R.A. and FLINT, D.J. (1986). Adipose tissue metabolism in sheep : Response to season and its modulation by reproductive state. Hormone and Metabolic Research 18, 308-312.

VERNON, R.G. and FLINT, D.J. (1984). Adipose tissue : Metabolic adaptation during lactation. In: Physiological Strategies in Lactation (Eds. M. Peaker, R.G. Vernon and C.H. Knight) Symposia of the Zoological Society of London No. 51 Academic Press, London 119-145.

WALLACE, L.R. (1948). The growth of lambs before and after birth in relation to the level of nutrition. Journal of Agricultural Science, Cambridge 38, 93-153.

WALLACE, A.L.C. and BASSETT, J.M. (1966). Effect of sheep growth hormone on plasma insulin concetration in sheep. Metabolism 15 95-97.

WALSH, D.S., VESELY, J.A. and MAHADEVANS, S. (1980). Relationship between milk production and circulating hormones in dairy cows. Journal of Dairy Science 63, 290-294.

WEEKES, T.E.C. and GODDEN, P.M.M. (1981). Nutrition and metabolic hormones. In: Hormones and Metabolism in Ruminants. ARC Workshop Proceedings, 99-111.

WEST, C.E., BICKERSTAFFE, R., ANNISON, E.F. and LINZELL, J.L. (1972). Studies on the mode of uptake of blood triglycerides by the mammary gland of the lactating goat : The uptake and incorporation into milk fat and mammary lymph of labelled glycerol, fatty acids and triglycerides. Biochemical Journal 126, 477-490.

WILCOX, A.A., CARROLL, W.E., STERLING, R.E., DAVIS, H.A. and WARE, A.G. (1966). Use of the Berthelot reaction in automated analysis of serum urea nitrogen. Clinical Chemistry 12 151-157.

ZIVIN, J.A. and SNARR, J.F. (1973). An automated colorimetric method for the measurement of 3-hydroxybutyrate concentration. Analytical Biochemistry 52, 456-461.